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Purified hepatitis C virus envelope proteins for diagnostic and therapeutic use

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ABSTRACT

The present invention relates to a method for purifying recombinant HCV single or specific oligomeric envelope proteins selected from the group consisting of E1 and/or E2 and/or E1/E2, characterized in that upon lysing the transformed host cells to isolate
5 the recombinantly expressed protein a disulphide bond cleavage or reduction step is carried out with a disulphide bond cleavage agent. The present invention also relates to a composition isolated by such a method. The present invention also relates to the diagnostic and therapeutic application of these compositions. Furthermore, the invention relates to the use of HCV E1 protein and peptides for prognosing and monitoring the
10 clinical effectiveness and/or clinical outcome of HCV treatment.

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Invention Title: 'PURIFIED HEPATITIS C VIRUS ENVELOPE PROTEINS FOR
DIAGNOSTIC AND THERAPEUTIC USE'

Details of Original Application No. 33824/95 dated 31 JUL 1995

The following statement is a full description of this invention, including the best method of performing it known to me/us:-

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**PURIFIED HEPATITIS C VIRUS ENVELOPE PROTEINS FOR DIAGNOSTIC
AND THERAPEUTIC USE**

Field of the invention

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The present invention relates to the general fields of recombinant protein expression, purification of recombinant proteins, synthetic peptides, diagnosis of HCV infection, prophylactic treatment against HCV infection and to the prognosis/monitoring of the clinical efficiency of treatment of an individual with chronic hepatitis, or the
10 prognosis/monitoring of natural disease.

More particularly, the present invention relates to purification methods for hepatitis C virus envelope proteins, the use in diagnosis, prophylaxis or therapy of HCV envelope proteins purified according to the methods described in the present invention, the use of single or specific oligomeric E1 and/or E2 and/or E1/E2 envelope proteins in assays for
15 monitoring disease, and/or diagnosis of disease, and/or treatment of disease. The invention also relates to epitopes of the E1 and/or E2 envelope proteins and monoclonal antibodies thereto, as well their use in diagnosis, prophylaxis or treatment.

Background of the invention

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The E2 protein purified from cell lysates according to the methods described in the present invention reacts with approximately 95% of patient sera. This reactivity is similar to the reactivity obtained with E2 secreted from CHO cells (Spaete et al., 1992). However, the intracellularly expressed form of E2 may more closely resemble the native viral
25 envelope protein because it contains high mannose carbohydrate motifs, whereas the E2 protein secreted from CHO cells is further modified with galactose and sialic acid sugar moieties. When the aminoterminal half of E2 is expressed in the baculovirus system, only about 13 to 21% of sera from several patient groups can be detected (Inoue et al., 1992). After expression of E2 from E. coli, the reactivity of HCV sera was even lower and ranged
30 from 14 (Yokosuka et al., 1992) to 17% (Mita et al., 1992).

About 75% of HCV sera (and 95% of chronic patients) are anti-E1 positive using the purified, vaccinia-expressed recombinant E1 protein of the present invention, in sharp contrast with the results of Kohara et al. (1992) and Hsu et al. (1993). Kohara et al. used a vaccinia-virus expressed E1 protein and detected anti-E1 antibodies in 7 to 23% of patients, while Hsu et al. only detected 14/50 (28%) sera using baculovirus-expressed E1.

These results show that not only a good expression system but also a good purification protocol are required to reach a high reactivity of the envelope proteins with human patient sera. This can be obtained using the proper expression system and/or purification protocols of the present invention which guarantee the conservation of the natural folding of the protein and the purification protocols of the present invention which guarantee the elimination of contaminating proteins and which preserve the conformation, and thus the reactivity of the HCV envelope proteins. The amounts of purified HCV envelope protein needed for diagnostic screening assays are in the range of grams per year. For vaccine purposes, even higher amounts of envelope protein would be needed. Therefore, the vaccinia virus system may be used for selecting the best expression constructs and for limited upscaling, and large-scale expression and purification of single or specific oligomeric envelope proteins containing high-mannose carbohydrates may be achieved when expressed from several yeast strains. In the case of hepatitis B for example, manufacturing of HBsAg from mammalian cells was much more costly compared with yeast-derived hepatitis B vaccines.

Aims of the invention

It is an aim of the present invention to provide a new purification method for recombinantly expressed E1 and/or E2 and/or E1/E2 proteins such that said recombinant proteins are directly usable for diagnostic and vaccine purposes as single or specific oligomeric recombinant proteins free from contaminants instead of aggregates.

It is another aim of the present invention to provide compositions comprising purified (single or specific oligomeric) recombinant E1 and/or E2 and/or E1/E2

glycoproteins comprising conformational epitopes from the E1 and/or E2 domains of HCV.

It is yet another aim of the present invention to provide novel recombinant vector constructs for recombinantly expressing E1 and/or E2 and/or E1/E2 proteins, as well as
5 host cells transformed with said vector constructs.

It is also an aim of the present invention to provide a method for producing and purifying recombinant HCV E1 and/or E2 and/or E1/E2 proteins.

It is also an aim of the present invention to provide diagnostic and immunogenic uses of the recombinant HCV E1 and/or E2 and/or E1/E2 proteins of the present invention,
10 as well as to provide kits for diagnostic use, vaccines or therapeutics comprising any of the recombinant HCV E1 and/or E2 and/or E1/E2 proteins of the present invention.

It is further an aim of the present invention to provide for a new use of E1, E2, and/or E1/E2 proteins, or suitable parts thereof, for monitoring/prognosing the response to treatment of patients (e.g. with interferon) suffering from HCV infection.

It is also an aim of the present invention to provide for the use of the recombinant E1, E2, and/or E1/E2 proteins of the present invention in HCV screening and confirmatory antibody tests.
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It is also an aim of the present invention to provide E1 and/or E2 peptides which can be used for diagnosis of HCV infection and for raising antibodies. Such peptides may
20 also be used to isolate human monoclonal antibodies.

It is also an aim of the present invention to provide monoclonal antibodies, more particularly human monoclonal antibodies or mouse monoclonal antibodies which are humanized, which react specifically with E1 and/or E2 epitopes, either comprised in peptides or conformational epitopes comprised in recombinant proteins.

It is also an aim of the present invention to provide possible uses of anti-E1 or anti-E2 monoclonal antibodies for HCV antigen detection or for therapy of chronic HCV infection.
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It is also an aim of the present invention to provide kits for monitoring/prognosing the response to treatment (e.g. with interferon) of patients suffering from HCV infection or
30 monitoring/prognosing the outcome of the disease.

All the aims of the present invention are considered to have been met by the embodiments as set out below.

Definitions

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The following definitions serve to illustrate the different terms and expressions used in the present invention.

The term 'hepatitis C virus single envelope protein' refers to a polypeptide or an analogue thereof (e.g. mimotopes) comprising an amino acid sequence (and/or amino acid analogues) defining at least one HCV epitope of either the E1 or the E2 region. These single envelope proteins in the broad sense of the word may be both monomeric or homo-oligomeric forms of recombinantly expressed envelope proteins. Typically, the sequences defining the epitope correspond to the amino acid sequence of either the E1 or the E2 region of HCV (either identically or via substitution of analogues of the native amino acid residue that do not destroy the epitope). In general, the epitope-defining sequence will be 3 or more amino acids in length, more typically, 5 or more amino acids in length, more typically 8 or more amino acids in length, and even more typically 10 or more amino acids in length. With respect to conformational epitopes, the length of the epitope-defining sequence can be subject to wide variations, since it is believed that these epitopes are formed by the three-dimensional shape of the antigen (e.g. folding). Thus, the amino acids defining the epitope can be relatively few in number, but widely dispersed along the length of the molecule being brought into the correct epitope conformation via folding. The portions of the antigen between the residues defining the epitope may not be critical to the conformational structure of the epitope. For example, deletion or substitution of these intervening sequences may not affect the conformational epitope provided sequences critical to epitope conformation are maintained (e.g. cysteines involved in disulfide bonding, glycosylation sites, etc.). A conformational epitope may also be formed by 2 or more essential regions of subunits of a homooligomer or heterooligomer.

The HCV antigens of the present invention comprise conformational epitopes from the E1 and/or E2 (envelope) domains of HCV. The E1 domain, which is believed to correspond to the viral envelope protein, is currently estimated to span amino acids 192-

383 of the HCV polyprotein (Hijikata et al., 1991). Upon expression in a mammalian system (glycosylated), it is believed to have an approximate molecular weight of 35 kDa as determined via SDS-PAGE. The E2 protein, previously called NS1, is believed to span amino acids 384-809 or 384-746 (Grakoui et al., 1993) of the HCV polyprotein and to also
5 be an envelope protein. Upon expression in a vaccinia system (glycosylated), it is believed to have an apparent gel molecular weight of about 72 kDa. It is understood that these protein endpoints are approximations (e.g. the carboxy terminal end of E2 could lie somewhere in the 730-820 amino acid region, e.g. ending at amino acid 730, 735, 740, 742, 744, 745, preferably 746, 747, 748, 750, 760, 770, 780, 790, 800, 809, 810, 820). The
10 E2 protein may also be expressed together with the E1, P7 (aa 747-809), NS2 (aa 810-1026), NS4A (aa 1658-1711) or NS4B (aa 1712-1972). Expression together with these other HCV proteins may be important for obtaining the correct protein folding.

It is also understood that the isolates used in the examples section of the present invention were not intended to limit the scope of the invention and that any HCV isolate
15 from type 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or any other new genotype of HCV is a suitable source of E1 and/or E2 sequence for the practice of the present invention.

The E1 and E2 antigens used in the present invention may be full-length viral proteins, substantially full-length versions thereof, or functional fragments thereof (e.g. fragments which are not missing sequence essential to the formation or retention of an
20 epitope). Furthermore, the HCV antigens of the present invention can also include other sequences that do not block or prevent the formation of the conformational epitope of interest. The presence or absence of a conformational epitope can be readily determined through screening the antigen of interest with an antibody (polyclonal serum or monoclonal to the conformational epitope) and comparing its reactivity to that of a denatured version of
25 the antigen which retains only linear epitopes (if any). In such screening using polyclonal antibodies, it may be advantageous to adsorb the polyclonal serum first with the denatured antigen and see if it retains antibodies to the antigen of interest.

The HCV antigens of the present invention can be made by any recombinant method that provides the epitope of interest. For example, recombinant intracellular
30 expression in mammalian or insect cells is a preferred method to provide glycosylated E1 and/or E2 antigens in 'native' conformation as is the case for the natural HCV antigens.

Yeast cells and mutant yeast strains (e.g. mnn 9 mutant (Kniskern et al., 1994) or glycosylation mutants derived by means of vanadate resistance selection (Ballou et al., 1991)) may be ideally suited for production of secreted high-mannose-type sugars; whereas proteins secreted from mammalian cells may contain modifications including galactose or sialic acids which may be undesirable for certain diagnostic or vaccine applications. However, it may also be possible and sufficient for certain applications, as it is known for proteins, to express the antigen in other recombinant hosts (such as *E. coli*) and renature the protein after recovery.

The term 'fusion polypeptide' intends a polypeptide in which the HCV antigen(s) are part of a single continuous chain of amino acids, which chain does not occur in nature. The HCV antigens may be connected directly to each other by peptide bonds or be separated by intervening amino acid sequences. The fusion polypeptides may also contain amino acid sequences exogenous to HCV.

The term 'solid phase' intends a solid body to which the individual HCV antigens or the fusion polypeptide comprised of HCV antigens are bound covalently or by noncovalent means such as hydrophobic adsorption.

The term 'biological sample' intends a fluid or tissue of a mammalian individual (e.g. an anthropoid, a human) that commonly contains antibodies produced by the individual, more particularly antibodies against HCV. The fluid or tissue may also contain HCV antigen. Such components are known in the art and include, without limitation, blood, plasma, serum, urine, spinal fluid, lymph fluid, secretions of the respiratory, intestinal or genitourinary tracts, tears, saliva, milk, white blood cells and myelomas. Body components include biological liquids. The term 'biological liquid' refers to a fluid obtained from an organism. Some biological fluids are used as a source of other products, such as clotting factors (e.g. Factor VIII;C), serum albumin, growth hormone and the like. In such cases, it is important that the source of biological fluid be free of contamination by virus such as HCV.

The term 'immunologically reactive' means that the antigen in question will react specifically with anti-HCV antibodies present in a body component from an HCV infected individual.

The term 'immune complex' intends the combination formed when an antibody binds to an epitope on an antigen.

'E1' as used herein refers to a protein or polypeptide expressed within the first 400 amino acids of an HCV polyprotein, sometimes referred to as the E, ENV or S protein. In its natural form it is a 35 kDa glycoprotein which is found in strong association with membranes. In most natural HCV strains, the E1 protein is encoded in the viral polyprotein following the C (core) protein. The E1 protein extends from approximately amino acid (aa) 192 to about aa 383 of the full-length polyprotein.

The term 'E1' as used herein also includes analogs and truncated forms that are immunologically cross-reactive with natural E1, and includes E1 proteins of genotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or any other newly identified HCV type or subtype.

'E2' as used herein refers to a protein or polypeptide expressed within the first 900 amino acids of an HCV polyprotein, sometimes referred to as the NS1 protein. In its natural form it is a 72 kDa glycoprotein that is found in strong association with membranes. In most natural HCV strains, the E2 protein is encoded in the viral polyprotein following the E1 protein. The E2 protein extends from approximately amino acid position 384 to amino acid position 746, another form of E2 extends to amino acid position 809.

The term 'E2' as used herein also includes analogs and truncated forms that are immunologically cross-reactive with natural E2. For example, insertions of multiple codons between codon 383 and 384, as well as deletions of amino acids 384-387 have been reported by Kato et al. (1992).

'E1/E2' as used herein refers to an oligomeric form of envelope proteins containing at least one E1 component and at least one E2 component.

The term 'specific oligomeric' E1 and/or E2 and/or E1/E2 envelope proteins refers to all possible oligomeric forms of recombinantly expressed E1 and/or E2 envelope proteins which are not aggregates. E1 and/or E2 specific oligomeric envelope proteins are also referred to as homo-oligomeric E1 or E2 envelope proteins (see below).

The term 'single or specific oligomeric' E1 and/or E2 and/or E1/E2 envelope proteins refers to single monomeric E1 or E2 proteins (single in the strict sense of the word) as well as specific oligomeric E1 and/or E2 and/or E1/E2 recombinantly expressed proteins. These single or specific oligomeric envelope proteins according to the present

invention can be further defined by the following formula $(E1)_x(E2)_y$, wherein x can be a number between 0 and 100, and y can be a number between 0 and 100, provided that x and y are not both 0. With x=1 and y=0 said envelope proteins include monomeric E1.

The term 'homo-oligomer' as used herein refers to a complex of E1 and/or E2
5 containing more than one E1 or E2 monomer, e.g. E1/E1 dimers, E1/E1/E1 trimers or
E1/E1/E1/E1 tetramers and E2/E2 dimers, E2/E2/E2 trimers or E2/E2/E2/E2 tetramers, E1
pentamers and hexamers, E2 pentamers and hexamers or any higher-order homo-oligomers
of E1 or E2 are all 'homo-oligomers' within the scope of this definition. The oligomers may
contain one, two, or several different monomers of E1 or E2 obtained from different types
10 or subtypes of hepatitis C virus including for example those described in an international
application published under WO 94/25601 and European application No. 94870166.9 both
by the present applicants. Such mixed oligomers are still homo-oligomers within the scope
of this invention, and may allow more universal diagnosis, prophylaxis or treatment of
HCV.

The term 'purified' as applied to proteins herein refers to a composition wherein the
15 desired protein comprises at least 35% of the total protein component in the composition.
The desired protein preferably comprises at least 40%, more preferably at least about 50%,
more preferably at least about 60%, still more preferably at least about 70%, even more
preferably at least about 80%, even more preferably at least about 90%, and most
20 preferably at least about 95% of the total protein component. The composition may contain
other compounds such as carbohydrates, salts, lipids, solvents, and the like, without
affecting the determination of the percentage purity as used herein. An 'isolated' HCV
protein intends an HCV protein composition that is at least 35% pure.

The term 'essentially purified proteins' refers to proteins purified such that they can
25 be used for in vitro diagnostic methods and as a therapeutic compound. These proteins are
substantially free from cellular proteins, vector-derived proteins or other HCV viral
components. Usually these proteins are purified to homogeneity (at least 80% pure,
preferably, 90%, more preferably 95%, more preferably 97%, more preferably 98%, more
preferably 99%, even more preferably 99.5%, and most preferably the contaminating
30 proteins should be undetectable by conventional methods like SDS-PAGE and silver
staining.

The term 'recombinantly expressed' used within the context of the present invention refers to the fact that the proteins of the present invention are produced by recombinant expression methods be it in prokaryotes, or lower or higher eukaryotes as discussed in detail below.

5 The term 'lower eukaryote' refers to host cells such as yeast, fungi and the like. Lower eukaryotes are generally (but not necessarily) unicellular. Preferred lower eukaryotes are yeasts, particularly species within Saccharomyces, Schizosaccharomyces, Kluveromyces, Pichia (e.g. Pichia pastoris), Hansenula (e.g. Hansenula polymorpha), Yarrowia, Schwaniomyces, Schizosaccharomyces, Zygosaccharomyces and the like.
10 Saccharomyces cerevisiae, S. carlsbergensis and K. lactis are the most commonly used yeast hosts, and are convenient fungal hosts.

 The term 'prokaryotes' refers to hosts such as E.coli, Lactobacillus, Lactococcus, Salmonella, Streptococcus, Bacillus subtilis or Streptomyces. Also these hosts are contemplated within the present invention.

15 The term 'higher eukaryote' refers to host cells derived from higher animals, such as mammals, reptiles, insects, and the like. Presently preferred higher eukaryote host cells are derived from Chinese hamster (e.g. CHO), monkey (e.g. COS and Vero cells), baby hamster kidney (BHK), pig kidney (PK15), rabbit kidney 13 cells (RK13), the human osteosarcoma cell line 143 B, the human cell line HeLa and human hepatoma cell lines like
20 Hep G2, and insect cell lines (e.g. Spodoptera frugiperda). The host cells may be provided in suspension or flask cultures, tissue cultures, organ cultures and the like. Alternatively the host cells may also be transgenic animals.

 The term 'polypeptide' refers to a polymer of amino acids and does not refer to a specific length of the product; thus, peptides, oligopeptides, and proteins are included
25 within the definition of polypeptide. This term also does not refer to or exclude post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. Included within the definition are, for example, polypeptides containing one or more analogues of an amino acid (including, for example, unnatural amino acids, PNA, etc.), polypeptides with substituted linkages, as well as other
30 modifications known in the art, both naturally occurring and non-naturally occurring.

The term 'recombinant polynucleotide or nucleic acid' intends a polynucleotide or nucleic acid of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation : (1) is not associated with all or a portion of a polynucleotide with which it is associated in nature, (2) is linked to a polynucleotide other than that to which it is linked in nature, or (3) does not occur in nature.

The term 'recombinant host cells', 'host cells', 'cells', 'cell lines', 'cell cultures', and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities refer to cells which can be or have been, used as recipients for a recombinant vector or other transfer polynucleotide, and include the progeny of the original cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation.

The term 'replicon' is any genetic element, e.g., a plasmid, a chromosome, a virus, a cosmid, etc., that behaves as an autonomous unit of polynucleotide replication within a cell; i.e., capable of replication under its own control.

The term 'vector' is a replicon further comprising sequences providing replication and/or expression of a desired open reading frame.

The term 'control sequence' refers to polynucleotide sequences which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and terminators; in eukaryotes, generally, such control sequences include promoters, terminators and, in some instances, enhancers. The term 'control sequences' is intended to include, at a minimum, all components whose presence is necessary for expression, and may also include additional components whose presence is advantageous, for example, leader sequences which govern secretion.

The term 'promoter' is a nucleotide sequence which is comprised of consensus sequences which allow the binding of RNA polymerase to the DNA template in a manner such that mRNA production initiates at the normal transcription initiation site for the adjacent structural gene.

The expression 'operably linked' refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence 'operably linked' to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

An 'open reading frame' (ORF) is a region of a polynucleotide sequence which encodes a polypeptide and does not contain stop codons; this region may represent a portion of a coding sequence or a total coding sequence.

A 'coding sequence' is a polynucleotide sequence which is transcribed into mRNA and/or translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include but is not limited to mRNA, DNA (including cDNA), and recombinant polynucleotide sequences.

As used herein, 'epitope' or 'antigenic determinant' means an amino acid sequence that is immunoreactive. Generally an epitope consists of at least 3 to 4 amino acids, and more usually, consists of at least 5 or 6 amino acids, sometimes the epitope consists of about 7 to 8, or even about 10 amino acids. As used herein, an epitope of a designated polypeptide denotes epitopes with the same amino acid sequence as the epitope in the designated polypeptide, and immunologic equivalents thereof. Such equivalents also include strain, subtype (=genotype), or type(group)-specific variants, e.g. of the currently known sequences or strains belonging to genotypes 1a, 1b, 1c, 1d, 1e, 1f, 2a, 2b, 2c, 2d, 2e, 2f, 2g, 2h, 2i, 3a, 3b, 3c, 3d, 3e, 3f, 3g, 4a, 4b, 4c, 4d, 4e, 4f, 4g, 4h, 4i, 4j, 4k, 4l, 5a, 5b, 6a, 6b, 6c, 7a, 7b, 7c, 8a, 8b, 9a, 9b, 10a, or any other newly defined HCV (sub)type. It is to be understood that the amino acids constituting the epitope need not be part of a linear sequence, but may be interspersed by any number of amino acids, thus forming a conformational epitope.

The term 'immunogenic' refers to the ability of a substance to cause a humoral and/or cellular response, whether alone or when linked to a carrier, in the presence or absence of an adjuvant. 'Neutralization' refers to an immune response that blocks the infectivity, either partially or fully, of an infectious agent. A 'vaccine' is an immunogenic

composition capable of eliciting protection against HCV, whether partial or complete. A vaccine may also be useful for treatment of an individual, in which case it is called a therapeutic vaccine.

The term 'therapeutic' refers to a composition capable of treating HCV infection.

5 The term 'effective amount' refers to an amount of epitope-bearing polypeptide sufficient to induce an immunogenic response in the individual to which it is administered, or to otherwise detectably immunoreact in its intended system (e.g., immunoassay). Preferably, the effective amount is sufficient to effect treatment, as defined above. The exact amount necessary will vary according to the application. For vaccine applications or
10 for the generation of polyclonal antiserum / antibodies, for example, the effective amount may vary depending on the species, age, and general condition of the individual, the severity of the condition being treated, the particular polypeptide selected and its mode of administration, etc. It is also believed that effective amounts will be found within a relatively large, non-critical range. An appropriate effective amount can be readily
15 determined using only routine experimentation. Preferred ranges of E1 and/or E2 and/or E1/E2 single or specific oligomeric envelope proteins for prophylaxis of HCV disease are 0.01 to 100 µg/dose, preferably 0.1 to 50 µg/dose. Several doses may be needed per individual in order to achieve a sufficient immune response and subsequent protection against HCV disease.

20

Detailed description of the invention

More particularly, the present invention contemplates a method for isolating or
25 purifying recombinant HCV single or specific oligomeric envelope protein selected from the group consisting of E1 and/or E2 and/or E1/E2, characterized in that upon lysing the transformed host cells to isolate the recombinantly expressed protein a disulphide bond cleavage or reduction step is carried out with a disulphide bond cleaving agent.

The essence of these 'single or specific oligomeric' envelope proteins of the
30 invention is that they are free from contaminating proteins and that they are not disulphide bond linked with contaminants.

The proteins according to the present invention are recombinantly expressed in lower or higher eukaryotic cells or in prokaryotes. The recombinant proteins of the present invention are preferably glycosylated and may contain high-mannose-type, hybrid, or complex glycosylations. Preferentially said proteins are expressed from mammalian cell lines as discussed in detail in the Examples section, or in yeast such as in mutant yeast strains also as detailed in the Examples section.

The proteins according to the present invention may be secreted or expressed within components of the cell, such as the ER or the Golgi Apparatus. Preferably, however, the proteins of the present invention bear high-mannose-type glycosylations and are retained in the ER or Golgi Apparatus of mammalian cells or are retained in or secreted from yeast cells, preferably secreted from yeast mutant strains such as the *mn9* mutant (Kniskern et al., 1994), or from mutants that have been selected by means of vanadate resistance (Ballou et al., 1991).

Upon expression of HCV envelope proteins, the present inventors could show that some of the free thiol groups of cysteines not involved in intra- or inter-molecular disulphide bridges, react with cysteines of host or expression-system-derived (e.g. vaccinia) proteins or of other HCV envelope proteins (single or oligomeric), and form aspecific intermolecular bridges. This results in the formation of 'aggregates' of HCV envelope proteins together with contaminating proteins. It was also shown in WO 92/08734 that 'aggregates' were obtained after purification, but it was not described which protein interactions were involved. In patent application WO 92/08734, recombinant E1/E2 protein expressed with the vaccinia virus system were partially purified as aggregates and only found to be 70% pure, rendering the purified aggregates not useful for diagnostic, prophylactic or therapeutic purposes.

Therefore, a major aim of the present invention resides in the separation of single or specific-oligomeric HCV envelope proteins from contaminating proteins, and to use the purified proteins (> 95% pure) for diagnostic, prophylactic and therapeutic purposes. To those purposes, the present inventors have been able to provide evidence that aggregated protein complexes ('aggregates') are formed on the basis of disulphide bridges and non-covalent protein-protein interactions. The present invention thus provides a means for selectively cleaving the disulphide bonds under specific conditions and for separating the

cleaved proteins from contaminating proteins which greatly interfere with diagnostic, prophylactic and therapeutic applications. The free thiol groups may be blocked (reversibly or irreversibly) in order to prevent the reformation of disulphide bridges, or may be left to oxidize and oligomerize with other envelope proteins (see definition homo-oligomer). It is to be understood that such protein oligomers are essentially different from the 'aggregates' described in WO 92/08734 and WO 94/01778, since the level of contaminating proteins is undetectable.

Said disulphide bond cleavage may also be achieved by:

- (1) performic acid oxidation by means of cysteic acid in which case the cysteine residues are modified into cysteic acid (Moore et al., 1963).
- (2) Sulfitolysis ($R-S-S-R \rightarrow 2 R-SO_3^-$) for example by means of sulphite (SO_3^{2-}) together with a proper oxidant such as Cu^{2+} in which case the cysteine is modified into S-sulpho-cysteine (Bailey and Cole, 1959).
- (3) Reduction by means of mercaptans, such as dithiotreitol (DTT), β -mercapto-ethanol, cysteine, glutathione Red, ϵ -mercapto-ethylamine, or thioglycolic acid, of which DTT and β -mercapto-ethanol are commonly used (Cleland, 1964), is the preferred method of this invention because the method can be performed in a water environment and because the cysteine remains unmodified.
- (4) Reduction by means of a phosphine (e.g. Bu_3P) (Ruegg and Rudinger, 1977).

All these compounds are thus to be regarded as agents or means for cleaving disulphide bonds according to the present invention.

Said disulphide bond cleavage (or reducing) step of the present invention is preferably a partial disulphide bond cleavage (reducing) step (carried out under partial cleavage or reducing conditions).

A preferred disulphide bond cleavage or reducing agent according to the present invention is dithiothreitol (DTT). Partial reduction is obtained by using a low concentration of said reducing agent, i.e. for DTT for example in the concentration range of about 0.1 to about 50 mM, preferably about 0.1 to about 20 mM, preferably about 0.5 to about 10 mM, preferably more than 1 mM, more than 2 mM or more than 5 mM, more preferably about 1.5 mM, about 2.0 mM, about 2.5 mM, about 5 mM or about 7.5 mM.

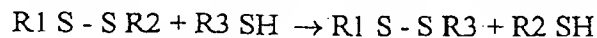
Said disulphide bond cleavage step may also be carried out in the presence of a suitable detergent (as an example of a means for cleaving disulphide bonds or in combination with a cleaving agent) able to dissociate the expressed proteins, such as DecylPEG, EMPIGEN-BB, NP-40, sodium cholate, Triton X-100.

5 Said reduction or cleavage step (preferably a partial reduction or cleavage step) is carried out preferably in the presence of (with) a detergent. A preferred detergent according to the present invention is Empigen-BB. The amount of detergent used is preferably in the range of 1 to 10 %, preferably more than 3%, more preferably about 3.5% of a detergent such as Empigen-BB.

10 A particularly preferred method for obtaining disulphide bond cleavage employs a combination of a classical disulphide bond cleavage agent as detailed above and a detergent (also as detailed above). As contemplated in the Examples section, the particular combination of a low concentration of DTT (1.5 to 7.5 mM) and about 3.5 % of Empigen-BB is proven to be a particularly preferred combination of reducing agent and detergent for
15 the purification of recombinantly expressed E1 and E2 proteins. Upon gelfiltration chromatography, said partial reduction is shown to result in the production of possibly dimeric E1 protein and separation of this E1-protein from contaminating proteins that cause false reactivity upon use in immunoassays.

It is, however, to be understood that also any other combination of any reducing
20 agent known in the art with any detergent or other means known in the art to make the cysteines better accessible is also within the scope of the present invention, insofar as said combination reaches the same goal of disulphide bridge cleavage as the preferred combination exemplified in the present invention.

Apart from reducing the disulphide bonds, a disulphide bond cleaving means
25 according to the present invention may also include any disulphide bridge exchanging agents (competitive agent being either organic or proteinaeous, see for instance Creighton, 1988) known in the art which allows the following type of reaction to occur:



* R1, R2: compounds of protein aggregates

* R3 SH: competitive agent (organic, proteinaeous)

30

The term 'disulphide bridge exchanging agent' is to be interpreted as including disulphide bond reforming as well as disulphide bond blocking agents.

The present invention also relates to methods for purifying or isolating HCV single or specific oligomeric envelope proteins as set out above further including the use of any
5 SH group blocking or binding reagent known in the art such as chosen from the following list:

- Glutathion
- 5,5'-dithiobis-(2-nitrobenzoic acid) or bis-(3-carboxy-4-nitrophenyl)-disulphide (DTNB or Ellman's reagent) (Elmann, 1959)
- 10 - N-ethylmaleimide (NEM; Benesch et al., 1956)
- N-(4-dimethylamino-3,5-dinitrophenyl) maleimide or Tuppy's maleimide which provides a color to the protein
- P-chloromercuribenzoate (Grassetti et al., 1969)
- 4-vinylpyridine (Friedman and Krull, 1969) can be liberated after reaction by acid
15 hydrolysis
- acrylonitrile, can be liberated after reaction by acid hydrolysis (Weil and Seibles, 1961)
- NEM-biotin (e.g. obtained from Sigma B1267)
- 2,2'-dithiopyridine (Grassetti and Murray, 1967)
- 20 - 4,4'-dithiopyridine (Grassetti and Murray, 1967)
- 6,6'-dithiodinicotinic acid (DTDNA; Brown and Cunningham, 1970)
- 2,2'-dithiobis-(5'-nitropyridine) (DTNP; US patent 3597160) or other dithiobis (heterocyclic derivative) compounds (Grassetti and Murray, 1969)

A survey of the publications cited shows that often different reagents for sulphydryl
25 groups will react with varying numbers of thiol groups of the same protein or enzyme molecule. One may conclude that this variation in reactivity of the thiol groups is due to the steric environment of these groups, such as the shape of the molecule and the surrounding groups of atoms and their charges, as well as to the size, shape and charge of the reagent molecule or ion. Frequently the presence of adequate concentrations of
30 denaturants such as sodium dodecylsulfate, urea or guanidine hydrochloride will cause sufficient unfolding of the protein molecule to permit equal access to all of the reagents for

thiol groups. By varying the concentration of denaturant, the degree of unfolding can be controlled and in this way thiol groups with different degrees of reactivity may be revealed. Although up to date most of the work reported has been done with p-chloromercuribenzoate, N-ethylmaleimide and DTNB, it is likely that the other more recently developed reagents may prove equally useful. Because of their varying structures, it seems likely, in fact, that they may respond differently to changes in the steric environment of the thiol groups.

Alternatively, conditions such as low pH (preferably lower than pH 6) for preventing free SH groups from oxidizing and thus preventing the formation of large intermolecular aggregates upon recombinant expression and purification of E1 and E2 (envelope) proteins are also within the scope of the present invention.

A preferred SH group blocking reagent according to the present invention is N-ethylmaleimide (NEM). Said SH group blocking reagent may be administered during lysis of the recombinant host cells and after the above-mentioned partial reduction process or after any other process for cleaving disulphide bridges. Said SH group blocking reagent may also be modified with any group capable of providing a detectable label and/or any group aiding in the immobilization of said recombinant protein to a solid substrate, e.g., biotinylated NEM.

Methods for cleaving cysteine bridges and blocking free cysteines have also been described in Darbre (1987), Means and Feeney (1971), and by Wong (1993).

A method to purify single or specific oligomeric recombinant E1 and/or E2 and/or E1/E2 proteins according to the present invention as defined above is further characterized as comprising the following steps:

- lysing recombinant E1 and/or E2 and/or E1/E2 expressing host cells, preferably in the presence of an SH group blocking agent, such as N-ethylmaleimide (NEM), and possibly a suitable detergent, preferably Empigen-BB,
- recovering said HCV envelope protein by affinity purification for instance by means lectin-chromatography, such as lentil-lectin chromatography, or immunoaffinity chromatography using anti-E1 and/or anti-E2 specific monoclonal antibodies, followed by,

- reduction or cleavage of disulphide bonds with a disulphide bond cleaving agent, such as DTT, preferably also in the presence of an SH group blocking agent, such as NEM or Biotin-NEM, and,
- recovering the reduced HCV E1 and/or E2 and/or E1/E2 envelope proteins for instance by gelfiltration (size exclusion chromatography or molecular sieving) and possibly also by an additional Ni^{2+} -IMAC chromatography and desalting step.

It is to be understood that the above-mentioned recovery steps may also be carried out using any other suitable technique known by the person skilled in the art.

Preferred lectin-chromatography systems include Galanthus nivalis agglutinin (GNA) - chromatography, or Lens culinaris agglutinin (LCA) (lentil) lectin chromatography as illustrated in the Examples section. Other useful lectins include those recognizing high-mannose type sugars, such as Narcissus pseudonarcissus agglutinin (NPA), Pisum sativum agglutinin (PSA), or Allium ursinum agglutinin (AUA).

Preferably said method is usable to purify single or specific oligomeric HCV envelope protein produced intracellularly as detailed above.

For secreted E1 or E2 or E1/E2 oligomers, lectins binding complex sugars such as Ricinus communis agglutinin I (RCA I), are preferred lectins.

The present invention more particularly contemplates essentially purified recombinant HCV single or specific oligomeric envelope proteins, selected from the group consisting of E1 and/or E2 and/or E1/E2, characterized as being isolated or purified by a method as defined above.

The present invention more particularly relates to the purification or isolation of recombinant envelope proteins which are expressed from recombinant mammalian cells such as vaccinia.

The present invention also relates to the purification or isolation of recombinant envelope proteins which are expressed from recombinant yeast cells.

The present invention equally relates to the purification or isolation of recombinant envelope proteins which are expressed from recombinant bacterial (prokaryotic) cells.

The present invention also contemplates a recombinant vector comprising a vector sequence, an appropriate prokaryotic, eukaryotic or viral or synthetic promoter sequence

followed by a nucleotide sequence allowing the expression of the single or specific oligomeric E1 and/or E2 and/or E1/E2 of the invention.

Particularly, the present invention contemplates a recombinant vector comprising a vector sequence, an appropriate prokaryotic, eukaryotic or viral or synthetic promoter
5 sequence followed by a nucleotide sequence allowing the expression of the single E1 or E1 of the invention.

Particularly, the present invention contemplates a recombinant vector comprising a vector sequence, an appropriate prokaryotic, eukaryotic or viral or synthetic promoter
10 sequence followed by a nucleotide sequence allowing the expression of the single E1 or E2 of the invention.

The segment of the HCV cDNA encoding the desired E1 and/or E2 sequence inserted into the vector sequence may be attached to a signal sequence. Said signal sequence may be that from a non-HCV source, e.g. the IgG or tissue plasminogen activator (tpa) leader sequence for expression in mammalian cells, or the α -mating factor sequence
15 for expression into yeast cells; but particularly preferred constructs according to the present invention contain signal sequences appearing in the HCV genome before the respective start points of the E1 and E2 proteins. The segment of the HCV cDNA encoding the desired E1 and/or E2 sequence inserted into the vector may also include deletions e.g. of the hydrophobic domain(s) as illustrated in the examples section, or of the E2
20 hypervariable region I.

More particularly, the recombinant vectors according to the present invention encompass a nucleic acid having an HCV cDNA segment encoding the polyprotein starting in the region between amino acid positions 1 and 192 and ending in the region between positions 250 and 400 of the HCV polyprotein, more preferably ending in the
25 region between positions 250 and 341, even more preferably ending in the region between positions 290 and 341 for expression of the HCV single E1 protein. Most preferably, the present recombinant vector encompasses a recombinant nucleic acid having a HCV cDNA segment encoding part of the HCV polyprotein starting in the region between positions 117 and 192, and ending at any position in the region between positions 263 and 326, for
30 expression of HCV single E1 protein. Also within the scope of the present invention are forms that have the first hydrophobic domain deleted (positions 264 to 293 plus or minus 8

amino acids), or forms to which a 5'-terminal ATG codon and a 3'-terminal stop codon has been added, or forms which have a factor Xa cleavage site and/or 3 to 10, preferably 6 Histidine codons have been added.

More particularly, the recombinant vectors according to the present invention
5 encompass a nucleic acid having an HCV cDNA segment encoding the polyprotein starting in the region between amino acid positions 290 and 406 and ending in the region between positions 600 and 820 of the HCV polyprotein, more preferably starting in the region between positions 322 and 406, even more preferably starting in the region between positions 347 and 406, even still more preferably starting in the region between positions
10 364 and 406 for expression of the HCV single E2 protein. Most preferably, the present recombinant vector encompasses a recombinant nucleic acid having a HCV cDNA segment encoding the polyprotein starting in the region between positions 290 and 406, and ending at any position of positions 623, 650, 661, 673, 710, 715, 720, 746 or 809, for expression of HCV single E2 protein. Also within the scope of the present invention are
15 forms to which a 5'-terminal ATG codon and a 3'-terminal stop codon has been added, or forms which have a factor Xa cleavage site and/or 3 to 10, preferably 6 Histidine codons have been added.

A variety of vectors may be used to obtain recombinant expression of HCV single or specific oligomeric envelope proteins of the present invention. Lower eukaryotes such
20 as yeasts and glycosylation mutant strains are typically transformed with plasmids, or are transformed with a recombinant virus. The vectors may replicate within the host independently, or may integrate into the host cell genome.

Higher eukaryotes may be transformed with vectors, or may be infected with a recombinant virus, for example a recombinant vaccinia virus. Techniques and vectors for
25 the insertion of foreign DNA into vaccinia virus are well known in the art, and utilize, for example homologous recombination. A wide variety of viral promoter sequences, possibly terminator sequences and poly(A)-addition sequences, possibly enhancer sequences and possibly amplification sequences, all required for the mammalian expression, are available in the art. Vaccinia is particularly preferred since vaccinia halts the expression of host cell
30 proteins. Vaccinia is also very much preferred since it allows the expression of E1 and E2 proteins of HCV in cells or individuals which are immunized with the live recombinant

vaccinia virus. For vaccination of humans the avipox and Ankara Modified Virus (AMV) are particularly useful vectors.

Also known are insect expression transfer vectors derived from baculovirus Autographa californica nuclear polyhedrosis virus (AcNPV), which is a helper-
5 independent viral expression vector. Expression vectors derived from this system usually use the strong viral polyhedrin gene promoter to drive the expression of heterologous genes. Different vectors as well as methods for the introduction of heterologous DNA into the desired site of baculovirus are available to the man skilled in the art for baculovirus expression. Also different signals for posttranslational modification recognized by insect
10 cells are known in the art.

Also included within the scope of the present invention is a method for producing purified recombinant single or specific oligomeric HCV E1 or E2 or E1/E2 proteins, wherein the cysteine residues involved in aggregates formation are replaced at the level of the nucleic acid sequence by other residues such that aggregate formation is prevented.
15 The recombinant proteins expressed by recombinant vectors carrying such a mutated E1 and/or E2 protein encoding nucleic acid are also within the scope of the present invention.

The present invention also relates to recombinant E1 and/or E2 and/or E1/E2 proteins characterized in that at least one of their glycosylation sites has been removed and are consequently termed glycosylation mutants. As explained in the Examples section,
20 different glycosylation mutants may be desired to diagnose (screening, confirmation, prognosis, etc.) and prevent HCV disease according to the patient in question. An E2 protein glycosylation mutant lacking the GLY4 has for instance been found to improve the reactivity of certain sera in diagnosis. These glycosylation mutants are preferably purified according to the method disclosed in the present invention. Also contemplated within the
25 present invention are recombinant vectors carrying the nucleic acid insert encoding such a E1 and/or E2 and/or E1/E2 glycosylation mutant as well as host cells transformed with such a recombinant vector.

The present invention also relates to recombinant vectors including a polynucleotide which also forms part of the present invention. The present invention
30 relates more particularly to the recombinant nucleic acids as represented in SEQ ID NO 3, 5, 7, 9, 11, 13, 21, 23, 25, 27, 29, 31, 35, 37, 39, 41, 43, 45, 47 and 49, or parts thereof.

The present invention also contemplates host cells transformed with a recombinant vector as defined above, wherein said vector comprises a nucleotide sequence encoding HCV E1 and/or E2 and/or E1/E2 protein as defined above in addition to a regulatory sequence operably linked to said HCV E1 and/or E2 and/or E1/E2 sequence and capable of
5 regulating the expression of said HCV E1 and/or E2 and/or E1/E2 protein.

Eukaryotic hosts include lower and higher eukaryotic hosts as described in the definitions section. Lower eukaryotic hosts include yeast cells well known in the art. Higher eukaryotic hosts mainly include mammalian cell lines known in the art and include many immortalized cell lines available from the ATCC, including HeLa cells, Chinese
10 hamster ovary (CHO) cells, Baby hamster kidney (BHK) cells, PK15, RK13 and a number of other cell lines.

The present invention relates particularly to a recombinant E1 and/or E2 and/or E1/E2 protein expressed by a host cell as defined above containing a recombinant vector as defined above. These recombinant proteins are particularly purified according to the
15 method of the present invention.

A preferred method for isolating or purifying HCV envelope proteins as defined above is further characterized as comprising at least the following steps:

- growing a host cell as defined above transformed with a recombinant vector according to the present invention or with a known recombinant vector expressing
20 E1 and/or E2 and/or E1/E2 HCV envelope proteins in a suitable culture medium,
- causing expression of said vector sequence as defined above under suitable conditions, and,
- lysing said transformed host cells, preferably in the presence of a SH group blocking agent, such as N-ethylmaleimide (NEM), and possibly a suitable
25 detergent, preferably Empigen-BB,
- recovering said HCV envelope protein by affinity purification such as by means of lectin-chromatography or immunoaffinity chromatography using anti-E1 and/or anti-E2 specific monoclonal antibodies, with said lectin being preferably lentil-lectin or GNA, followed by,

- incubation of the eluate of the previous step with a disulphide bond cleavage means, such as DTT, preferably followed by incubation with an SH group blocking agent, such as NEM or Biotin-NEM, and,
- isolating the HCV single or specific oligomeric E1 and/or E2 and/or E1/E2 proteins such as by means of gelfiltration and possibly also by a subsequent Ni^{2+} -IMAC chromatography followed by a desalting step.

As a result of the above-mentioned proces, E1 and/or E2 and/or E1/E2 proteins may be produced in a form which elute differently from the large aggregates containing vector-derived components and/or cell components in the void volume of the gelfiltration column or the IMAC column as illustrated in the Examples section. The disulphide bridge cleavage step advantageously also eliminates the false reactivity due to the presence of host and/or expression-system-derived proteins. The presence of NEM and a suitable detergent during lysis of the cells may already partly or even completely prevent the aggregation between the HCV envelope proteins and contaminants.

Ni^{2+} -IMAC chromatography followed by a desalting step is preferably used for constructs bearing a $(\text{His})_6$ as described by Janknecht et al., 1991, and Hochuli et al., 1988.

The present invention also relates to a method for producing monoclonal antibodies in small animals such as mice or rats, as well as a method for screening and isolating human B-cells that recognize anti-HCV antibodies, using the HCV single or specific oligomeric envelope proteins of the present invention.

The present invention further relates to a composition comprising at least one of the following E1 peptides as listed in Table 3:

- E1-31 (SEQ ID NO 56) spanning amino acids 181 to 200 of the Core/E1 V1 region,
- E1-33 (SEQ ID NO 57) spanning amino acids 193 to 212 of the E1 region,
- E1-35 (SEQ ID NO 58) spanning amino acids 205 to 224 of the E1 V2 region (epitope B),
- E1-35A (SEQ ID NO 59) spanning amino acids 208 to 227 of the E1 V2 region (epitope B),
- 1bE1 (SEQ ID NO 53) spanning amino acids 192 to 228 of E1 regions (V1, C1, and V2 regions (containing epitope B)),

E1-51 (SEQ ID NO 66) spanning amino acids 301 to 320 of the E1 region,
E1-53 (SEQ ID NO 67) spanning amino acids 313 to 332 of the E1 C4 region
(epitope A),
E1-55 (SEQ ID NO 68) spanning amino acids 325 to 344 of the E1 region.

5 The present invention also relates to a composition comprising at least one of the
following E2 peptides as listed in Table 3:

Env 67 or E2-67 (SEQ ID NO 72) spanning amino acid positions 397 to 416 of the
E2 region (epitope A, recognized by monoclonal antibody 2F10H10, see Figure
19),

10 Env 69 or E2-69 (SEQ ID NO 73) spanning amino acid positions 409 to 428 of the
E2 region (epitope A),

Env 23 or E2-23 (SEQ ID NO 86) spanning positions 583 to 602 of the E2 region
(epitope E),

15 Env 25 or E2-25 (SEQ ID NO 87) spanning positions 595 to 614 of the E2 region
(epitope E),

Env 27 or E2-27 (SEQ ID NO 88) spanning positions 607 to 626 of the E2 region
(epitope E),

Env 17B or E2-17B (SEQ ID NO 83) spanning positions 547 to 566 of the E2
region (epitope D),

20 Env 13B or E2-13B (SEQ ID NO 82) spanning positions 523 to 542 of the E2
region (epitope C; recognized by monoclonal antibody 16A6E7, see Figure 19).

The present invention also relates to a composition comprising at least one of the
following E2 conformational epitopes:

25 epitope F recognized by monoclonal antibodies 15C8C1, 12D11F1 and
8G10D1H9,

epitope G recognized by monoclonal antibody 9G3E6,

epitope H (or C) recognized by monoclonal antibody 10D3C4 and 4H6B2, or,

epitope I recognized by monoclonal antibody 17F2C2.

30 The present invention also relates to an E1 or E2 specific antibody raised upon
immunization with a peptide or protein composition, with said antibody being specifically

reactive with any of the polypeptides or peptides as defined above, and with said antibody being preferably a monoclonal antibody.

The present invention also relates to an E1 or E2 specific antibody screened from a variable chain library in plasmids or phages or from a population of human B-cells by means of a process known in the art, with said antibody being reactive with any of the polypeptides or peptides as defined above, and with said antibody being preferably a monoclonal antibody.

The E1 or E2 specific monoclonal antibodies of the invention can be produced by any hybridoma liable to be formed according to classical methods from splenic cells of an animal, particularly from a mouse or rat, immunized against the HCV polypeptides or peptides according to the invention, as defined above on the one hand, and of cells of a myeloma cell line on the other hand, and to be selected by the ability of the hybridoma to produce the monoclonal antibodies recognizing the polypeptides which has been initially used for the immunization of the animals.

The antibodies involved in the invention can be labelled by an appropriate label of the enzymatic, fluorescent, or radioactive type.

The monoclonal antibodies according to this preferred embodiment of the invention may be humanized versions of mouse monoclonal antibodies made by means of recombinant DNA technology, departing from parts of mouse and/or human genomic DNA sequences coding for H and L chains from cDNA or genomic clones coding for H and L chains.

Alternatively the monoclonal antibodies according to this preferred embodiment of the invention may be human monoclonal antibodies. These antibodies according to the present embodiment of the invention can also be derived from human peripheral blood lymphocytes of patients infected with HCV, or vaccinated against HCV. Such human monoclonal antibodies are prepared, for instance, by means of human peripheral blood lymphocytes (PBL) repopulation of severe combined immune deficiency (SCID) mice (for recent review, see Duchosal et al., 1992).

The invention also relates to the use of the proteins or peptides of the invention, for the selection of recombinant antibodies by the process of repertoire cloning (Persson et al., 1991).

Antibodies directed to peptides or single or specific oligomeric envelope proteins derived from a certain genotype may be used as a medicament, more particularly for incorporation into an immunoassay for the detection of HCV genotypes (for detecting the presence of HCV E1 or E2 antigen), for prognosing/monitoring of HCV disease, or as
5 therapeutic agents.

Alternatively, the present invention also relates to the use of any of the above-specified E1 or E2 specific monoclonal antibodies for the preparation of an immunoassay kit for detecting the presence of E1 or E2 antigen in a biological sample, for the preparation of a kit for prognosing/monitoring of HCV disease or for the preparation of a HCV
10 medicament.

The present invention also relates to the a method for *in vitro* diagnosis or detection of HCV antigen present in a biological sample, comprising at least the following steps :

- (i) contacting said biological sample with any of the E1 and/or E2 specific monoclonal antibodies as defined above, preferably in an immobilized form
15 under appropriate conditions which allow the formation of an immune complex,
- (ii) removing unbound components,
- (iii) incubating the immune complexes formed with heterologous antibodies, which specifically bind to the antibodies present in the sample to be
20 analyzed, with said heterologous antibodies having conjugated to a detectable label under appropriate conditions,
- (iv) detecting the presence of said immune complexes visually or mechanically (e.g. by means of densitometry, fluorimetry, colorimetry).

The present invention also relates to a kit for *in vitro* diagnosis of HCV antigen
25 present in a biological sample, comprising:

- at least one monoclonal antibody as defined above, with said antibody being preferentially immobilized on a solid substrate,
- a buffer or components necessary for producing the buffer enabling binding
30 reaction between these antibodies and the HCV antigens present in the biological sample,

- a means for detecting the immune complexes formed in the preceding binding reaction,
- possibly also including an automated scanning and interpretation device for inferring the HCV antigens present in the sample from the observed binding pattern.

5

The present invention also relates to a composition comprising E1 and/or E2 and/or E1/E2 recombinant HCV proteins purified according to the method of the present invention or a composition comprising at least one peptides as specified above for use as a medicament.

10

The present invention more particularly relates to a composition comprising at least one of the above-specified envelope peptides or a recombinant envelope protein composition as defined above, for use as a vaccine for immunizing a mammal, preferably humans, against HCV, comprising administering a sufficient amount of the composition possibly accompanied by pharmaceutically acceptable adjuvant(s), to produce an immune response.

15

More particularly, the present invention relates to the use of any of the compositions as described here above for the preparation of a vaccine as described above.

20

Also, the present invention relates to a vaccine composition for immunizing a mammal, preferably humans, against HCV, comprising HCV single or specific oligomeric proteins or peptides derived from the E1 and/or the E2 region as described above.

25

Immunogenic compositions can be prepared according to methods known in the art. The present compositions comprise an immunogenic amount of a recombinant E1 and/or E2 and/or E1/E2 single or specific oligomeric proteins as defined above or E1 or E2 peptides as defined above, usually combined with a pharmaceutically acceptable carrier, preferably further comprising an adjuvant.

30

The single or specific oligomeric envelope proteins of the present invention, either E1 and/or E2 and/or E1/E2, are expected to provide a particularly useful vaccine antigen, since the formation of antibodies to either E1 or E2 may be more desirable than to the other envelope protein, and since the E2 protein is cross-reactive between HCV types and the E1 protein is type-specific. Cocktails including type 1 E2 protein and E1 proteins derived from several genotypes may be particularly advantageous. Cocktails containing a molar excess

of E1 versus E2 or E2 versus E1 may also be particularly useful. Immunogenic compositions may be administered to animals to induce production of antibodies, either to provide a source of antibodies or to induce protective immunity in the animal.

Pharmaceutically acceptable carriers include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers; and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to : aluminim hydroxide (alum), N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP) as found in U.S. Patent No. 4,606,918, N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE) and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate, and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. Any of the 3 components MPL, TDM or CWS may also be used alone or combined 2 by 2. Additionally, adjuvants such as Stimulon (Cambridge Bioscience, Worcester, MA) or SAF-1 (Syntex) may be used. Further, Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA) may be used for non-human applications and research purposes.

The immunogenic compositions typically will contain pharmaceutically acceptable vehicles, such as water, saline, glycerol, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, preservatives, and the like, may be included in such vehicles.

Typically, the immunogenic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect. The E1 and E2 proteins may also be incorporated into Immune Stimulating Complexes together with saponins, for example Quil A (ISCOMS).

Immunogenic compositions used as vaccines comprise a 'sufficient amount' or 'an immunologically effective amount' of the envelope proteins of the present invention, as well as any other of the above mentioned components, as needed. 'Immunologically effective amount', means that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment, as defined above. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated (e.g. nonhuman primate, primate, etc.), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, the strain of infecting HCV, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials. Usually, the amount will vary from 0.01 to 1000 $\mu\text{g}/\text{dose}$, more particularly from 0.1 to 100 $\mu\text{g}/\text{dose}$.

The single or specific oligomeric envelope proteins may also serve as vaccine carriers to present homologous (e.g. T cell epitopes or B cell epitopes from the core, NS2, NS3, NS4 or NS5 regions) or heterologous (non-HCV) haptens, in the same manner as Hepatitis B surface antigen (see European Patent Application 174,444). In this use, envelope proteins provide an immunogenic carrier capable of stimulating an immune response to haptens or antigens conjugated to the aggregate. The antigen may be conjugated either by conventional chemical methods, or may be cloned into the gene encoding E1 and/or E2 at a location corresponding to a hydrophilic region of the protein. Such hydrophilic regions include the V1 region (encompassing amino acid positions 191 to 202), the V2 region (encompassing amino acid positions 213 to 223), the V3 region (encompassing amino acid positions 230 to 242), the V4 region (encompassing amino acid positions 230 to 242), the V5 region (encompassing amino acid positions 294 to 303) and the V6 region (encompassing amino acid positions 329 to 336). Another useful location for insertion of haptens is the hydrophobic region (encompassing approximately amino acid positions 264 to 293). It is shown in the present invention that this region can be deleted without affecting the reactivity of the deleted E1 protein with antisera. Therefore, haptens may be inserted at the site of the deletion.

The immunogenic compositions are conventionally administered parenterally, typically by injection, for example, subcutaneously or intramuscularly. Additional formulations suitable for other methods of administration include oral formulations and suppositories. Dosage treatment may be a single dose schedule or a multiple dose schedule.

5 The vaccine may be administered in conjunction with other immunoregulatory agents.

The present invention also relates to a composition comprising peptides or polypeptides as described above, for *in vitro* detection of HCV antibodies present in a biological sample.

10 The present invention also relates to the use of a composition as described above for the preparation of an immunoassay kit for detecting HCV antibodies present in a biological sample.

The present invention also relates to a method for *in vitro* diagnosis of HCV antibodies present in a biological sample, comprising at least the following steps :

- 15 (i) contacting said biological sample with a composition comprising any of the envelope peptide or proteins as defined above, preferably in an immobilized form under appropriate conditions which allow the formation of an immune complex, wherein said peptide or protein can be a biotinylated peptide or protein which is covalently bound to a solid substrate by means of streptavidin or avidin complexes,
- 20 (ii) removing unbound components,
- (iii) incubating the immune complexes formed with heterologous antibodies, with said heterologous antibodies having conjugated to a detectable label under appropriate conditions,
- 25 (iv) detecting the presence of said immune complexes visually or mechanically (e.g. by means of densitometry, fluorimetry, colorimetry).

Alternatively, the present invention also relates to competition immunoassay formats in which recombinantly produced purified single or specific oligomeric protein E1 and/or E2 and/or E1/E2 proteins as disclosed above are used in combination with E1 and/or E2 peptides in order to compete for HCV antibodies present in a biological sample.

30 The present invention also relates to a kit for determining the presence of HCV antibodies, in a biological sample, comprising :

- at least one peptide or protein composition as defined above, possibly in combination with other polypeptides or peptides from HCV or other types of HCV, with said peptides or proteins being preferentially immobilized on a solid substrate, more preferably on different microwells of the same ELISA plate, and even more preferentially on one and the same membrane strip,
- a buffer or components necessary for producing the buffer enabling binding reaction between these polypeptides or peptides and the antibodies against HCV present in the biological sample,
- means for detecting the immune complexes formed in the preceding binding reaction,
- possibly also including an automated scanning and interpretation device for inferring the HCV genotypes present in the sample from the observed binding pattern.

The immunoassay methods according to the present invention utilize single or specific oligomeric antigens from the E1 and/or E2 domains that maintain linear (in case of peptides) and conformational epitopes (single or specific oligomeric proteins) recognized by antibodies in the sera from individuals infected with HCV. It is within the scope of the invention to use for instance single or specific oligomeric antigens, dimeric antigens, as well as combinations of single or specific oligomeric antigens. The HCV E1 and E2 antigens of the present invention may be employed in virtually any assay format that employs a known antigen to detect antibodies. Of course, a format that denatures the HCV conformational epitope should be avoided or adapted. A common feature of all of these assays is that the antigen is contacted with the body component suspected of containing HCV antibodies under conditions that permit the antigen to bind to any such antibody present in the component. Such conditions will typically be physiologic temperature, pH and ionic strength using an excess of antigen. The incubation of the antigen with the specimen is followed by detection of immune complexes comprised of the antigen.

Design of the immunoassays is subject to a great deal of variation, and many formats are known in the art. Protocols may, for example, use solid supports, or immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the

labels may be, for example, enzymatic, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the immune complex are also known; examples of which are assays which utilize biotin and avidin or streptavidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

5 The immunoassay may be, without limitation, in a heterogeneous or in a homogeneous format, and of a standard or competitive type. In a heterogeneous format, the polypeptide is typically bound to a solid matrix or support to facilitate separation of the sample from the polypeptide after incubation. Examples of solid supports that can be used are nitrocellulose (e.g., in membrane or microtiter well form), polyvinyl chloride (e.g., in
10 sheets or microtiter wells), polystyrene latex (e.g., in beads or microtiter plates, polyvinylidene fluoride (known as ImmunolonTM), diazotized paper, nylon membranes, activated beads, and Protein A beads. For example, Dynatech ImmunolonTM 1 or ImmunolonTM 2 microtiter plates or 0.25 inch polystyrene beads (Precision Plastic Ball) can be used in the heterogeneous format. The solid support containing the antigenic
15 polypeptides is typically washed after separating it from the test sample, and prior to detection of bound antibodies. Both standard and competitive formats are known in the art.

 In a homogeneous format, the test sample is incubated with the combination of antigens in solution. For example, it may be under conditions that will precipitate any antigen-antibody complexes which are formed. Both standard and competitive formats for
20 these assays are known in the art.

 In a standard format, the amount of HCV antibodies in the antibody-antigen complexes is directly monitored. This may be accomplished by determining whether labeled anti-xenogeneic (e.g. anti-human) antibodies which recognize an epitope on anti-HCV antibodies will bind due to complex formation. In a competitive format, the amount
25 of HCV antibodies in the sample is deduced by monitoring the competitive effect on the binding of a known amount of labeled antibody (or other competing ligand) in the complex.

 Complexes formed comprising anti-HCV antibody (or in the case of competitive assays, the amount of competing antibody) are detected by any of a number of known
30 techniques, depending on the format. For example, unlabeled HCV antibodies in the

complex may be detected using a conjugate of anti-xenogeneic Ig complexed with a label (e.g. an enzyme label).

In an immunoprecipitation or agglutination assay format the reaction between the HCV antigens and the antibody forms a network that precipitates from the solution or suspension and forms a visible layer or film of precipitate. If no anti-HCV antibody is present in the test specimen, no visible precipitate is formed.

There currently exist three specific types of particle agglutination (PA) assays. These assays are used for the detection of antibodies to various antigens when coated to a support. One type of this assay is the hemagglutination assay using red blood cells (RBCs) that are sensitized by passively adsorbing antigen (or antibody) to the RBC. The addition of specific antigen antibodies present in the body component, if any, causes the RBCs coated with the purified antigen to agglutinate.

To eliminate potential non-specific reactions in the hemagglutination assay, two artificial carriers may be used instead of RBC in the PA. The most common of these are latex particles. However, gelatin particles may also be used. The assays utilizing either of these carriers are based on passive agglutination of the particles coated with purified antigens.

The HCV single or specific oligomeric E1 and/or E2 and/or E1/E2 antigens of the present invention comprised of conformational epitopes will typically be packaged in the form of a kit for use in these immunoassays. The kit will normally contain in separate containers the native HCV antigen, control antibody formulations (positive and/or negative), labeled antibody when the assay format requires the same and signal generating reagents (e.g. enzyme substrate) if the label does not generate a signal directly. The native HCV antigen may be already bound to a solid matrix or separate with reagents for binding it to the matrix. Instructions (e.g. written, tape, CD-ROM, etc.) for carrying out the assay usually will be included in the kit.

Immunoassays that utilize the native HCV antigen are useful in screening blood for the preparation of a supply from which potentially infective HCV is lacking. The method for the preparation of the blood supply comprises the following steps. Reacting a body component, preferably blood or a blood component, from the individual donating blood with HCV E1 and/or E2 proteins of the present invention to allow an immunological

reaction between HCV antibodies, if any, and the HCV antigen. Detecting whether anti-HCV antibody - HCV antigen complexes are formed as a result of the reacting. Blood contributed to the blood supply is from donors that do not exhibit antibodies to the native HCV antigens, E1 or E2.

5 In cases of a positive reactivity to the HCV antigen, it is preferable to repeat the immunoassay to lessen the possibility of false positives. For example, in the large scale screening of blood for the production of blood products (e.g. blood transfusion, plasma, Factor VIII, immunoglobulin, etc.) 'screening' tests are typically formatted to increase sensitivity (to insure no contaminated blood passes) at the expense of specificity; i.e. the
10 false-positive rate is increased. Thus, it is typical to only defer for further testing those donors who are 'repeatedly reactive'; i.e. positive in two or more runs of the immunoassay on the donated sample. However, for confirmation of HCV-positivity, the 'confirmation' tests are typically formatted to increase specificity (to insure that no false-positive samples are confirmed) at the expense of sensitivity. Therefore the purification method described in
15 the present invention for E1 and E2 will be very advantageous for including single or specific oligomeric envelope proteins into HCV diagnostic assays.

The solid phase selected can include polymeric or glass beads, nitrocellulose, microparticles, microwells of a reaction tray, test tubes and magnetic beads. The signal generating compound can include an enzyme, a luminescent compound, a chromogen, a
20 radioactive element and a chemiluminescent compound. Examples of enzymes include alkaline phosphatase, horseradish peroxidase and beta-galactosidase. Examples of enhancer compounds include biotin, anti-biotin and avidin. Examples of enhancer compounds binding members include biotin, anti-biotin and avidin. In order to block the effects of rheumatoid factor-like substances, the test sample is subjected to conditions sufficient to
25 block the effect of rheumatoid factor-like substances. These conditions comprise contacting the test sample with a quantity of anti-human IgG to form a mixture, and incubating the mixture for a time and under conditions sufficient to form a reaction mixture product substantially free of rheumatoid factor-like substance.

The present invention further contemplates the use of E1 proteins, or parts thereof,
30 more particularly HCV single or specific oligomeric E1 proteins as defined above, for *in*

vitro monitoring HCV disease or prognosing the response to treatment (for instance with Interferon) of patients suffering from HCV infection comprising:

- incubating a biological sample from a patient with hepatitis C infection with an E1 protein or a suitable part thereof under conditions allowing the formation of an immunological complex,
- removing unbound components,
- calculating the anti-E1 titers present in said sample (for example at the start of and/or during the course of (interferon) therapy),
- monitoring the natural course of HCV disease, or prognosing the response to treatment of said patient on the basis of the amount anti-E1 titers found in said sample at the start of treatment and/or during the course of treatment.

Patients who show a decrease of 2, 3, 4, 5, 7, 10, 15, or preferably more than 20 times of the initial anti-E1 titers could be concluded to be long-term, sustained responders to HCV therapy, more particularly to interferon therapy. It is illustrated in the Examples section, that an anti-E1 assay may be very useful for prognosing long-term response to IFN treatment, or to treatment of Hepatitis C virus disease in general.

More particularly the following E1 peptides as listed in Table 3 were found to be useful for *in vitro* monitoring HCV disease or prognosing the response to interferon treatment of patients suffering from HCV infection:

- E1-31 (SEQ ID NO 56) spanning amino acids 181 to 200 of the Core/E1 V1 region,
- E1-33 (SEQ ID NO 57) spanning amino acids 193 to 212 of the E1 region,
- E1-35 (SEQ ID NO 58) spanning amino acids 205 to 224 of the E1 V2 region (epitope B),
- E1-35A (SEQ ID NO 59) spanning amino acids 208 to 227 of the E1 V2 region (epitope B),
- 1bE1 (SEQ ID NO 53) spanning amino acids 192 to 228 of E1 regions (V1, C1, and V2 regions (containing epitope B)),
- E1-51 (SEQ ID NO 66) spanning amino acids 301 to 320 of the E1 region,

E1-53 (SEQ ID NO 67) spanning amino acids 313 to 332 of the E1 C4 region (epitope A),

E1-55 (SEQ ID NO 68) spanning amino acids 325 to 344 of the E1 region.

It is to be understood that smaller fragments of the above-mentioned peptides also
5 fall within the scope of the present invention. Said smaller fragments can be easily prepared by chemical synthesis and can be tested for their ability to be used in an assay as detailed above and in the Examples section.

The present invention also relates to a kit for monitoring HCV disease or prognosing the response to treatment (for instance to interferon) of patients suffering from
10 HCV infection comprising:

- at least one E1 protein or E1 peptide, more particularly an E1 protein or E1 peptide as defined above,
- a buffer or components necessary for producing the buffer enabling the
15 binding reaction between these proteins or peptides and the anti-E1 antibodies present in a biological sample,
- means for detecting the immune complexes formed in the preceding binding reaction,
- possibly also an automated scanning and interpretation device for inferring a decrease of anti-E1 titers during the progression of treatment.

20 It is to be understood that also E2 protein and peptides according to the present invention can be used to a certain degree to monitor/prognose HCV treatment as indicated above for the E1 proteins or peptides because also the anti-E2 levels decrease in comparison to antibodies to the other HCV antigens. It is to be understood, however, that it might be possible to determine certain epitopes in the E2 region which would also be
25 suited for use in an test for monitoring/prognosing HCV disease.

The present invention also relates to a serotyping assay for detecting one or more serological types of HCV present in a biological sample, more particularly for detecting antibodies of the different types of HCV to be detected combined in one assay format, comprising at least the following steps :

- 30 (i) contacting the biological sample to be analyzed for the presence of HCV antibodies of one or more serological types, with at least one of the E1

and/or E2 and/or E1/E2 protein compositions or at least one of the E1 or E2 peptide compositions as defined above, preferentially in an immobilized form under appropriate conditions which allow the formation of an immune complex,

- 5 (ii) removing unbound components,
- (iii) incubating the immune complexes formed with heterologous antibodies, with said heterologous antibodies being conjugated to a detectable label under appropriate conditions,
- 10 (iv) detecting the presence of said immune complexes visually or mechanically (e.g. by means of densitometry, fluorimetry, colorimetry) and inferring the presence of one or more HCV serological types present from the observed binding pattern.

It is to be understood that the compositions of proteins or peptides used in this method are recombinantly expressed type-specific envelope proteins or type-specific peptides.

The present invention further relates to a kit for serotyping one or more serological types of HCV present in a biological sample, more particularly for detecting the antibodies to these serological types of HCV comprising:

- 20 - at least one E1 and/or E2 and/or E1/E2 protein or E1 or E2 peptide, as defined above,
- a buffer or components necessary for producing the buffer enabling the binding reaction between these proteins or peptides and the anti-E1 antibodies present in a biological sample,
- 25 - means for detecting the immune complexes formed in the preceding binding reaction,
- possibly also an automated scanning and interpretation device for detecting the presence of one or more serological types present from the observed binding pattern.

The present invention also relates to the use of a peptide or protein composition as defined above, for immobilization on a solid substrate and incorporation into a reversed phase hybridization assay, preferably for immobilization as parallel lines onto a solid

support such as a membrane strip, for determining the presence or the genotype of HCV according to a method as defined above. Combination with other type-specific antigens from other HCV polyprotein regions also lies within the scope of the present invention.

Figure and Table legends

5

Figure 1 : Restriction map of plasmid pgpt ATA 18

Figure 2 : Restriction map of plasmid pgs ATA 18

Figure 3 : Restriction map of plasmid pMS 66

Figure 4 : Restriction map of plasmid pv HCV-11A

10 Figure 5 : Anti-E1 levels in non-responders to IFN treatment

Figure 6 : Anti-E1 levels in responders to IFN treatment

Figure 7 : Anti-E1 levels in patients with complete response to IFN treatment

Figure 8 : Anti-E1 levels in incomplete responders to IFN treatment

Figure 9 : Anti-E2 levels in non-responders to IFN treatment

15 Figure 10 : Anti-E2 levels in responders to IFN treatment

Figure 11 : Anti-E2 levels in incomplete responders to IFN treatment

Figure 12 : Anti-E2 levels in complete responders to IFN treatment

Figure 13 : Human anti-E1 reactivity competed with peptides

Figure 14 : Competition of reactivity of anti-E1 monoclonal antibodies with peptides

20 Figure 15 : Anti-E1 (epitope 1) levels in non-responders to IFN treatment

Figure 16 : Anti-E1 (epitope 1) levels in responders to IFN treatment

Figure 17 : Anti-E1 (epitope 2) levels in non-responders to IFN treatment

Figure 18 : Anti-E1 (epitope 2) levels in responders to IFN treatment

Figure 19 : Competition of reactivity of anti-E2 monoclonal antibodies with peptides

25 Figure 20 : Human anti-E2 reactivity competed with peptides

Figure 21 : Nucleic acid sequences of the present invention. The nucleic acid sequences encoding an E1 or E2 protein according to the present invention may be translated (SEQ ID NO 3 to 13, 21-31, 35 and 41-49 are translated in a reading frame starting from residue number 1, SEQ ID NO 37-39 are translated in a reading frame starting from residue number 2), into the

30

amino acid sequences of the respective E1 or E2 proteins as shown in the sequence listing.

- Figure 22: ELISA results obtained from lentil lectin chromatography eluate fractions of 4 different E1 purifications of cell lysates infected with vvHCV39 (type 1b), vvHCV40 (type 1b), vvHCV62 (type 3a), and vvHCV63 (type 5a).
- Figure 23: Elution profiles obtained from the lentil lectin chromatography of the 4 different E1 constructs on the basis of the values as shown in Figure 22.
- Figure 24: ELISA results obtained from fractions obtained after gelfiltration chromatography of 4 different E1 purifications of cell lysates infected with vvHCV39 (type 1b), vvHCV40 (type 1b), vvHCV62 (type 3a), and vvHCV63 (type 5a).
- Figure 25: Profiles obtained from purifications of E1 proteins of type 1b (1), type 3a (2), and type 5a (3) (from RK13 cells infected with vvHCV39, vvHCV62, and vvHCV63, respectively; purified on lentil lectin and reduced as in example 5.2 - 5.3) and a standard (4). The peaks indicated with '1', '2', and '3', represent pure E1 protein peaks (see Figure 24, E1 reactivity mainly in fractions 26 to 30).
- Figure 26: Silver staining of an SDS-PAGE as described in example 4 of a raw lysate of E1 vvHCV40 (type 1b) (lane 1), pool 1 of the gelfiltration of vvHCV40 representing fractions 10 to 17 as shown in Figure 25 (lane 2), pool 2 of the gelfiltration of vvHCV40 representing fractions 18 to 25 as shown in Figure 25 (lane 3), and E1 pool (fractions 26 to 30) (lane 4).
- Figure 27: Streptavidine-alkaline phosphatase blot of the fractions of the gelfiltration of E1 constructs 39 (type 1b) and 62 (type 3a). The proteins were labelled with NEM-biotin. Lane 1: start gelfiltration construct 39, lane 2: fraction 26 construct 39, lane 3: fraction 27 construct 39, lane 4: fraction 28 construct 39, lane 5: fraction 29 construct 39, lane 6: fraction 30 construct 39, lane 7: fraction 31 construct 39, lane 8: molecular weight marker, lane 9: start gelfiltration construct 62, lane 10: fraction 26 construct 62, lane 11: fraction 27 construct 62, lane 12: fraction 28 construct 62, lane 13: fraction 29

construct 62, lane 14: fraction 30 construct 62, lane 15: fraction 31 construct 62.

Figure 28: Silver staining of an SDS-PAGE gel of the gelfiltration fractions of vvHCV-39 (E1s, type 1b) and vvHCV-62 (E1s, type 3a) run under identical conditions as Figure 26. Lane 1: start gelfiltration construct 39, lane 2: fraction 26 construct 39, lane 3: fraction 27 construct 39, lane 4: fraction 28 construct 39, lane 5: fraction 29 construct 39, lane 6: fraction 30 construct 39, lane 7 fraction 31 construct 39, lane 8: molecular weight marker, lane 9: start gelfiltration construct 62, lane 10: fraction 26 construct 62, lane 11: fraction 27 construct 62, lane 12: fraction 28 construct 62, lane 13: fraction 29 construct 62, lane 14: fraction 30 construct 62, lane 15: fraction 31 construct 62.

Figure 29: Western Blot analysis with anti-E1 mouse monoclonal antibody 5E1A10 giving a complete overview of the purification procedure. Lane 1: crude lysate, Lane 2: flow through of lentil chromatography, Lane 3: wash with Empigen BB after lentil chromatography, Lane 4: Eluate of lentil chromatography, Lane 5: Flow through during concentration of the lentil eluate, Lane 6: Pool of E1 after Size Exclusion Chromatography (gelfiltration).

Figure 30: OD₂₈₀ profile (continuous line) of the lentil lectin chromatography of E2 protein from RK13 cells infected with vvHCV44. The dotted line represents the E2 reactivity as detected by ELISA (as in example 6).

Figure 31A: OD₂₈₀ profile (continuous line) of the lentil-lectin gelfiltration chromatography E2 protein pool from RK13 cells infected with vvHCV44 in which the E2 pool is applied immediately on the gelfiltration column (non-reduced conditions). The dotted line represents the E2 reactivity as detected by ELISA (as in example 6).

Figure 31B: OD₂₈₀ profile (continuous line) of the lentil-lectin gelfiltration chromatography E2 protein pool from RK13 cells infected with vvHCV44 in which the E2 pool was reduced and blocked according to Example 5.3

(reduced conditions). The dotted line represents the E2 reactivity as detected by ELISA (as in example 6).

- Figure 32: Ni²⁺-IMAC chromatography and ELISA reactivity of the E2 protein as expressed from vvHCV44 after gelfiltration under reducing conditions as shown in Figure 31B.
- Figure 33: Silver staining of an SDS-PAGE of 0.5 µg of purified E2 protein recovered by a 200 mM imidazole elution step (lane 2) and a 30mM imidazole wash (lane 1) of the Ni²⁺-IMAC chromatography as shown in Figure 32.
- Figure 34: OD profiles of a desalting step of the purified E2 protein recovered by 200 mM imidazole as shown in Figure 33, intended to remove imidazole.
- Figure 35A: Antibody levels to the different HCV antigens (Core 1, Core 2, E2HCVR, NS3) for NR and LTR followed during treatment and over a period of 6 to 12 months after treatment determined by means of the LIAscan method. The average values are indicated by the curves with the open squares.
- Figure 35B: Antibody levels to the different HCV antigens (NS4, NS5, E1 and E2) for NR and LTR followed during treatment and over a period of 6 to 12 months after treatment determined by means of the LIAscan method. The average values are indicated by the curve with the open squares.
- Figure 36: Average E1 antibody (E1Ab) and E2 antibody (E2Ab) levels in the LTR and NR groups.
- Figure 37: Averages E1 antibody (E1Ab) levels for non-responders (NR) and long term responders (LTR) for type 1b and type 3a.
- Figure 38: Relative map positions of the anti-E2 monoclonal antibodies.
- Figure 39: Partial deglycosylation of HCV E1 envelope protein. The lysate of vvHCV10A-infected RK13 cells were incubated with different concentrations of glycosidases according to the manufacturer's instructions. Right panel: Glycopeptidase F (PNGase F). Left panel: Endoglycosidase H (Endo H).
- Figure 40: Partial deglycosylation of HCV E2 envelope proteins. The lysate of vvHCV64-infected (E2) and vvHCV41-infected (E2s)RK13 cells were

incubated with different concentrations of Glycopeptidase F (PNGase F) according to the manufacturer's instructions.

Figure 41: In vitro mutagenesis of HCV E1 glycoproteins. Map of the mutated sequences and the creation of new restriction sites.

5 Figure 42A: In vitro mutagenesis of HCV E1 glycoprotein (part 1). First step of PCR amplification.

Figure 42B: In vitro mutagenesis of HCV E1 glycoprotein (part 2). Overlap extension and nested PCR.

10 Figure 43: In vitro mutagenesis of HCV E1 glycoproteins. Map of the PCR mutated fragments (GLY-# and OVR-#) synthesized during the first step of amplification.

Figure 44A: Analysis of E1 glycoprotein mutants by Western blot expressed in HeLa (left) and RK13 (right) cells. Lane 1: wild type VV (vaccinia virus), Lane 2: original E1 protein (vvHCV-10A), Lane 3: E1 mutant Gly-1 (vvHCV-81),
15 Lane 4: E1 mutant Gly-2 (vvHCV-82), Lane 5: E1 mutant Gly-3 (vvHCV-83), Lane 6: E1 mutant Gly-4 (vvHCV-84), Lane 7: E1 mutant Gly-5 (vvHCV-85), Lane 8: E1 mutant Gly-6 (vvHCV-86).

Figure 44B: Analysis of E1 glycosylation mutant vaccinia viruses by PCR amplification/restriction. Lane 1: E1 (vvHCV-10A), *BspE* I, Lane 2:
20 E1.GLY-1 (vvHCV-81), *BspE* I, Lane 4: E1 (vvHCV-10A), *Sac* I, Lane 5: E1.GLY-2 (vvHCV-82), *Sac* I, Lane 7: E1 (vvHCV-10A), *Sac* I, Lane 8: E1.GLY-3 (vvHCV-83), *Sac* I, Lane 10: E1 (vvHCV-10A), *Stu* I, Lane 11: E1.GLY-4 (vvHCV-84), *Stu* I, Lane 13: E1 (vvHCV-10A), *Sma* I, Lane 14: E1.GLY-5 (vvHCV-85), *Sma* I, Lane 16: E1 (vvHCV-10A), *Stu* I, Lane 17:
25 E1.GLY-6 (vvHCV-86), *Stu* I, Lane 3 - 6 - 9 - 12 - 15 : Low Molecular Weight Marker, pBluescript SK+, *Msp* I.

Figure 45: SDS polyacrylamide gel electrophoresis of recombinant E2 expressed in S. cerevisiae. Inoculates were grown in leucine selective medium for 72 hrs. and diluted 1/15 in complete medium. After 10 days of culture at 28°C,
30 medium samples were taken. The equivalent of 200 µl of culture

supernatant concentrated by speedvac was loaded on the gel. Two independent transformants were analysed.

Figure 46: SDS polyacrylamide gel electrophoresis of recombinant E2 expressed in a glycosylation deficient *S. cerevisiae* mutant. Inoculae were grown in leucine selective medium for 72 hrs. and diluted 1/15 in complete medium. After 10 days of culture at 28°C, medium samples were taken. The equivalent of 350 µl of culture supernatant, concentrated by ion exchange chromatography, was loaded on the gel.

Table 1 : Features of the respective clones and primers used for amplification for constructing the different forms of the E1 protein as despected in Example 1.

Table 2 : Summary of Anti-E1 tests

Table 3 : Synthetic peptides for competition studies

Table 4: Changes of envelope antibody levels over time.

Table 5: Difference between LTR and NR

Table 6: Competition experiments between murine E2 monoclonal antibodies

Table 7: Primers for construction of E1 glycosylation mutants

Table 8: Analysis of E1 glycosylation mutants by ELISA

Example 1: Cloning and expression of the hepatitis C virus E1 protein

1. Construction of vaccinia virus recombination vectors

The pgptATA18 vaccinia recombination plasmid is a modified version of pATA18 (Stunnenberg et al, 1988) with an additional insertion containing the *E. coli* xanthine guanine phosphoribosyl transferase gene under the control of the vaccinia virus I3 intermediate promoter (Figure 1). The plasmid pgsATA18 was constructed by inserting an oligonucleotide linker with SEQ ID NO 1/94, containing stop codons in the three reading frames, into the Pst I and HindIII-cut pATA18 vector. This created an extra Pac I restriction site (Figure 2). The original HindIII site was not restored.

Oligonucleotide linker with SEQ ID NO 1/94:

5 5' G GCATGC AAGCTT AATTAATT 3'
 3' ACGTC CGTACG TTCGAA TTAATTAA TCGA 5'
 PstI SphI HindIII Pac I (HindIII)

10

In order to facilitate rapid and efficient purification by means of Ni^{2+} chelation of engineered histidine stretches fused to the recombinant proteins, the vaccinia recombination vector pMS66 was designed to express secreted proteins with an additional carboxy-terminal histidine tag. An oligonucleotide linker with SEQ ID NO 2/95, containing unique sites for 3 restriction enzymes generating blunt ends (Sma I, Stu I and Pml I/Bbr PI) was synthesized in such a way that the carboxy-terminal end of any cDNA could be inserted in frame with a sequence encoding the protease factor Xa cleavage site followed by a nucleotide sequence encoding 6 histidines and 2 stop codons (a new Pac I restriction site was also created downstream the 3'end). This oligonucleotide with SEQ ID NO 2/95 was introduced between the Xma I and Pst I sites of pgptATA18 (Figure 3).

20

Oligonucleotide linker with SEQ ID NO 2/95:

5' CCGGG GAGGCCTGCACGTGATCGAGGGCAGACACCATCACCACCATCACTAATAGTTAATTAA CTGCA 3'
3' C CTCCGGACGTGCACTAGCTCCCGTCTGTGGTAGTGGTGGTAGTGATTATCAATTAATT G

25

Xma I

Pst I

30

Example 2. Construction of HCV recombinant plasmids

2.1. Constructs encoding different forms of the E1 protein

5 Polymerase Chain Reaction (PCR) products were derived from the serum samples by RNA preparation and subsequent reverse-transcription and PCR as described previously (Stuyver et al., 1993b). Table 1 shows the features of the respective clones and the primers used for amplification. The PCR fragments were cloned into the Sma I-cut pSP72 (Promega) plasmids. The following clones were selected for insertion into vaccinia recombination vectors: HCC19A (SEQ ID NO 3), HCC110A (SEQ ID NO 5), HCC111A (SEQ ID NO 7), HCC112A (SEQ ID NO 9), HCC113A (SEQ ID NO 11), and HCC117A (SEQ ID NO 13) as depicted in Figure 21. cDNA fragments containing the E1-coding regions were cleaved by EcoRI and HindIII restriction from the respective pSP72 plasmids and inserted into the EcoRI/HindIII-cut pgptATA-18 vaccinia recombination vector (described in example 1), downstream of the 11K vaccinia virus late promoter. The respective plasmids were designated pvHCV-9A, pvHCV-10A, pvHCV-11A, pvHCV-12A, pvHCV-13A and pvHCV-17A, of which pvHCV-11A is shown in Figure 4.

2.2. Hydrophobic region E1 deletion mutants

20 Clone HCC137, containing a deletion of codons Asp264 to Val287 (nucleotides 790 to 861, region encoding hydrophobic domain I) was generated as follows: 2 PCR fragments were generated from clone HCC110A with primer sets HCPPr52 (SEQ ID NO 16)/HCPPr107 (SEQ ID NO 19) and HCPPr108 (SEQ ID NO 20)/HCPPr54 (SEQ ID NO 18). 25 These primers are shown in Figure 21. The two PCR fragments were purified from agarose gel after electrophoresis and 1 ng of each fragment was used together as template for PCR by means of primers HCPPr52 (SEQ ID NO 16) and HCPPr54 (SEQ ID NO 18). The resulting fragment was cloned into the Sma I-cut pSP72 vector and clones containing the deletion were readily identified because of the deletion of 24 codons (72 base pairs). 30 Plasmid pSP72HCC137 containing clone HCC137 (SEQ ID 15) was selected. A recombinant vaccinia plasmid containing the full-length E1 cDNA lacking hydrophobic

domain I was constructed by inserting the HCV sequence surrounding the deletion (fragment cleaved by Xma I and BamH I from the vector pSP72-HCC137) into the Xma I-Bam H I sites of the vaccinia plasmid pvHCV-10A. The resulting plasmid was named pvHCV-37. After confirmatory sequencing, the amino-terminal region containing the
5 internal deletion was isolated from this vector pvHCV-37 (cleavage by EcoR I and BstE II) and reinserted into the Eco RI and Bst EII-cut pvHCV-11A plasmid. This construct was expected to express an E1 protein with both hydrophobic domains deleted and was named pvHCV-38. The E1-coding region of clone HCC138 is represented by SEQ ID NO 23.

As the hydrophilic region at the E1 carboxyterminus (theoretically extending to
10 around amino acids 337-340) was not completely included in construct pvHCV-38, a larger E1 region lacking hydrophobic domain I was isolated from the pvHCV-37 plasmid by EcoR I/Bam HI cleavage and cloned into an EcoRI/BamHI-cut pgsATA-18 vector. The resulting plasmid was named pvHCV-39 and contained clone HCC139 (SEQ ID NO 25). The same fragment was cleaved from the pvHCV-37 vector by BamH I (of which the
15 sticky ends were filled with Klenow DNA Polymerase I (Boehringer)) and subsequently by EcoR I (5' cohesive end). This sequence was inserted into the EcoRI and Bbr PI-cut vector pMS-66. This resulted in clone HCC140 (SEQ ID NO 27) in plasmid pvHCV-40, containing a 6 histidine tail at its carboxy-terminal end.

20 2.3. E1 of other genotypes

Clone HCC162 (SEQ ID NO 29) was derived from a type 3a-infected patient with chronic hepatitis C (serum BR36, clone BR36-9-13, SEQ ID NO 19 in WO 94/25601, and see also Stuyver et al. 1993a) and HCC163 (SEQ ID NO 31) was derived from a type 5a-
25 infected child with post-transfusion hepatitis (serum BE95, clone PC-4-1, SEQ ID NO 45 in WO 94/25601).

2.4. E2 constructs

30 The HCV E2 PCR fragment 22 was obtained from serum BE11 (genotype 1b) by means of primers HCP109 (SEQ ID NO 33) and HCP172 (SEQ ID NO 34) using

techniques of RNA preparation, reverse-transcription and PCR, as described in Stuyver et al., 1993b, and the fragment was cloned into the Sma I-cut pSP72 vector. Clone HCC122A (SEQ ID NO 35) was cut with NcoI/AlwNI or by BamHI/AlwNI and the sticky ends of the fragments were blunted (NcoI and BamHI sites with Klenow DNA Polymerase I (Boehringer), and AlwNI with T4 DNA polymerase (Boehringer)). The BamHI/AlwNI cDNA fragment was then inserted into the vaccinia pgsATA-18 vector that had been linearized by EcoR I and Hind III cleavage and of which the cohesive ends had been filled with Klenow DNA Polymerase (Boehringer). The resulting plasmid was named pvHCV-41 and encoded the E2 region from amino acids Met347 to Gln673, including 37 amino acids (from Met347 to Gly383) of the E1 protein that can serve as signal sequence. The same HCV cDNA was inserted into the EcoR I and Bbr PI-cut vector pMS66, that had subsequently been blunt ended with Klenow DNA Polymerase. The resulting plasmid was named pvHCV-42 and also encoded amino acids 347 to 683. The NcoI/AlwNI fragment was inserted in a similar way into the same sites of pgsATA-18 (pvHCV-43) or pMS-66 vaccinia vectors (pvHCV-44). pvHCV-43 and pvHCV-44 encoded amino acids 364 to 673 of the HCV polyprotein, of which amino acids 364 to 383 were derived from the natural carboxyterminal region of the E1 protein encoding the signal sequence for E2, and amino acids 384 to 673 of the mature E2 protein.

2.5. Generation of recombinant HCV-vaccinia viruses

Rabbit kidney RK13 cells (ATCC CCL 37), human osteosarcoma 143B thymidine kinase deficient (TK-) (ATCC CRL 8303), HeLa (ATCC CCL 2), and Hep G2 (ATCC HB 8065) cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, Md, USA). The cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10 % foetal calf serum, and with Earle's salts (EMEM) for RK13 and 143 B (TK-), and with glucose (4 g/l) for Hep G2. The vaccinia virus WR strain (Western Reserve, ATTC VR119) was routinely propagated in either 143B or RK13 cells, as described previously (Panicali & Paoletti, 1982; Piccini et al., 1987; Mackett et al., 1982, 1984, and 1986). A confluent monolayer of 143B cells was infected with wild type vaccinia virus at a multiplicity of infection (m.o.i.) of 0.1 (= 0.1 plaque forming unit (PFU)

per cell). Two hours later, the vaccinia recombination plasmid was transfected into the infected cells in the form of a calcium phosphate coprecipitate containing 500 ng of the plasmid DNA to allow homologous recombination (Graham & van der Eb, 1973; Mackett et al., 1985). Recombinant viruses expressing the *Escherichia coli* xanthine-guanine phosphoribosyl transferase (gpt) protein were selected on rabbit kidney RK13 cells incubated in selection medium (EMEM containing 25 µg/ml mycophenolic acid (MPA), 250 µg/ml xanthine, and 15 µg/ml hypoxanthine; Falkner and Moss, 1988; Janknecht et al, 1991). Single recombinant viruses were purified on fresh monolayers of RK13 cells under a 0.9% agarose overlay in selection medium. Thymidine kinase deficient (TK⁻) recombinant viruses were selected and then plaque purified on fresh monolayers of human 143B cells (TK⁻) in the presence of 25 µg/ml 5-bromo-2'-deoxyuridine. Stocks of purified recombinant HCV-vaccinia viruses were prepared by infecting either human 143 B or rabbit RK13 cells at an m.o.i. of 0.05 (Mackett et al, 1988). The insertion of the HCV cDNA fragment in the recombinant vaccinia viruses was confirmed on an aliquot (50 µl) of the cell lysate after the MPA selection by means of PCR with the primers used to clone the respective HCV fragments (see Table 1). The recombinant vaccinia-HCV viruses were named according to the vaccinia recombination plasmid number, e.g. the recombinant vaccinia virus vvHCV-10A was derived from recombining the wild type WR strain with the pvHCV-10A plasmid.

Example 3: infection of cells with recombinant vaccinia viruses

A confluent monolayer of RK13 cells was infected at a m.o.i. of 3 with the recombinant HCV-vaccinia viruses as described in example 2 . For infection, the cell monolayer was washed twice with phosphate-buffered saline pH 7.4 (PBS) and the recombinant vaccinia virus stock was diluted in MEM medium. Two hundred µl of the virus solution was added per 10⁶ cells such that the m.o.i. was 3, and incubated for 45 min at 24°C. The virus solution was aspirated and 2 ml of complete growth medium (see example 2) was added per 10⁶ cells. The cells were incubated for 24 hr at 37°C during which expression of the HCV proteins took place.

Example 4: Analysis of recombinant proteins by means of western blotting

5 The infected cells were washed two times with PBS, directly lysed with lysis buffer
(50 mM Tris.HCl pH 7.5, 150 mM NaCl, 1% Triton X-100, 5 mM MgCl₂, 1 µg/ml
aprotinin (Sigma, Bornem, Belgium)) or detached from the flasks by incubation in 50 mM
Tris.HCl pH 7.5/ 10 mM EDTA/ 150 mM NaCl for 5 min, and collected by centrifugation
(5 min at 1000g). The cell pellet was then resuspended in 200 µl lysis buffer (50 mM
10 Tris.HCl pH 8.0, 2 mM EDTA, 150 mM NaCl, 5 mM MgCl₂, aprotinin, 1% Triton X-
100) per 10⁶ cells. The cell lysates were cleared for 5 min at 14,000 rpm in an Eppendorf
centrifuge to remove the insoluble debris. Proteins of 20 µl lysate were separated by means
of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins
were then electro-transferred from the gel to a nitrocellulose sheet (Amersham) using a
15 Hoefer HSI transfer unit cooled to 4°C for 2 hr at 100 V constant voltage, in transfer buffer
(25 mM Tris.HCl pH 8.0, 192 mM glycine, 20% (v/v) methanol). Nitrocellulose filters
were blocked with Blotto (5 % (w/v) fat-free instant milk powder in PBS; Johnson et al.,
1981) and incubated with primary antibodies diluted in Blotto/0.1 % Tween 20. Usually, a
human negative control serum or serum of a patient infected with HCV were 200 times
20 diluted and preincubated for 1 hour at room temperature with 200 times diluted wild type
vaccinia virus-infected cell lysate in order to decrease the non-specific binding. After
washing with Blotto/0.1% Tween 20, the nitrocellulose filters were incubated with alkaline
phosphatase substrate solution diluted in Blotto/0.1 % Tween 20. After washing with 0.1%
Tween 20 in PBS, the filters were incubated with alkaline phosphatase substrate solution
25 (100 mM Tris.HCl pH 9.5, 100 mM NaCl, 5 mM MgCl₂, 0,38 µg/ml nitroblue tetrazolium,
0.165 µg/ml 5-bromo-4-chloro-3-indolylphosphate). All steps, except the electrotransfer,
were performed at room temperature.

Example 5: Purification of recombinant E1 or E2 protein

5.1. Lysis

5 Infected RK13 cells (carrying E1 or E2 constructs) were washed 2 times with phosphate-buffered saline (PBS) and detached from the culture recipients by incubation in PBS containing 10 mM EDTA. The detached cells were washed twice with PBS and 1 ml of lysis buffer (50 mM Tris.HCl pH 7.5, 150 mM NaCl, 1% Triton X-100, 5 mM MgCl₂, 1 µg/ml aprotinin (Sigma, Bornem, Belgium) containing 2 mM biotinylated N-ethylmaleimide (biotin-NEM) (Sigma) was added per 10⁵ cells at 4°C. This lysate was
10 homogenized with a type B douncer and left at room temperature for 0.5 hours. Another 5 volumes of lysis buffer containing 10 mM N-ethylmaleimide (NEM, Aldrich, Bornem, Belgium) was added to the primary lysate and the mixture was left at room temperature for 15 min. Insoluble cell debris was cleared from the solution by centrifugation in a Beckman
15 JA-14 rotor at 14,000 rpm (30100 g at r_{max}) for 1 hour at 4°C.

5.2. Lectin Chromatography

 The cleared cell lysate was loaded at a rate of 1ml/min on a 0.8 by 10 cm Lentil-
20 lectin Sepharose 4B column (Pharmacia) that had been equilibrated with 5 column volumes of lysis buffer at a rate of 1 ml/min. The lentil-lectin column was washed with 5 to 10 column volumes of buffer 1 (0.1M potassium phosphate pH 7.3, 500 mM KCl, 5% glycerol, 1 mM 6-NH₂-hexanoic acid, 1 mM MgCl₂, and 1% DecylPEG (KWANT, Bedum, The Netherlands). In some experiments, the column was subsequently washed
25 with 10 column volumes of buffer 1 containing 0.5% Empigen-BB (Calbiochem, San Diego, CA, USA) instead of 1% DecylPEG. The bound material was eluted by applying elution buffer (10 mM potassium phosphate pH 7.3, 5% glycerol, 1 mM hexanoic acid, 1mM MgCl₂, 0.5% Empigen-BB, and 0.5 M α-methyl-mannopyranoside). The eluted
30 material was fractionated and fractions were screened for the presence of E1 or E2 protein by means of ELISA as described in example 6. Figure 22 shows ELISA results obtained from lentil lectin eluate fractions of 4 different E1 purifications of cell lysates infected with

vvHCV39 (type 1b), vvHCV40 (type 1b), vvHCV62 (type 3a), and vvHCV63 (type 5a). Figure 23 shows the profiles obtained from the values shown in Figure 22. These results show that the lectin affinity column can be employed for envelope proteins of the different types of HCV.

5.3. Concentration and partial reduction

The E1- or E2-positive fractions were pooled and concentrated on a Centricon 30 kDa (Amicon) by centrifugation for 3 hours at 5,000 rpm in a Beckman JA-20 rotor at 4°C. In some experiments the E1- or E2-positive fractions were pooled and concentrated by nitrogen evaporation. An equivalent of $3 \cdot 10^8$ cells was concentrated to approximately 200 µl. For partial reduction, 30% Empigen-BB (Calbiochem, San Diego, CA, USA) was added to this 200 µl to a final concentration of 3.5 %, and 1M DTT in H₂O was subsequently added to a final concentration of 1.5 to 7.5 mM and incubated for 30 min at 37 °C. NEM (1M in dimethylsulphoxide) was subsequently added to a final concentration of 50 mM and left to react for another 30 min at 37°C to block the free sulphydryl groups.

5.4. Gel filtration chromatography

A Superdex-200 HR 10/20 column (Pharmacia) was equilibrated with 3 column volumes PBS/3% Empigen-BB. The reduced mixture was injected in a 500 µl sample loop of the Smart System (Pharmacia) and PBS/3% Empigen-BB buffer was added for gelfiltration. Fractions of 250 µl were collected from V_0 to V_t . The fractions were screened for the presence of E1 or E2 protein as described in example 6.

Figure 24 shows ELISA results obtained from fractions obtained after gelfiltration chromatography of 4 different E1 purifications of cell lysates infected with vvHCV39 (type 1b), vvHCV40 (type 1b), vvHCV62 (type 3a), and vvHCV63 (type 5a). Figure 25 shows the profiles obtained from purifications of E1 proteins of types 1b, 3a, and 5a (from RK13 cells infected with vvHCV39, vvHCV62, and vvHCV63, respectively; purified on lentil lectin and reduced as in the previous examples). The peaks indicated with '1', '2', and '3', represent pure E1 protein peaks (E1 reactivity mainly in fractions 26 to 30). These

peaks show very similar molecular weights of approximately 70 kDa, corresponding to dimeric E1 protein. Other peaks in the three profiles represent vaccinia virus and/or cellular proteins which could be separated from E1 only because of the reduction step as outlined in example 5.3. and because of the subsequent gelfiltration step in the presence of the proper detergent. As shown in Figure 26 pool 1 (representing fractions 10 to 17) and pool 2 (representing fractions 18 to 25) contain contaminating proteins not present in the E1 pool (fractions 26 to 30). The E1 peak fractions were ran on SDS/PAGE and blotted as described in example 4. Proteins labelled with NEM-biotin were detected by streptavidin-alkaline phosphatase as shown in Figure 27. It can be readily observed that, amongst others, the 29 kDa and 45kDa contaminating proteins present before the gelfiltration chromatography (lane 1) are only present at very low levels in the fractions 26 to 30. The band at approximately 65kDa represents the E1 dimeric form that could not be entirely disrupted into the monomeric E1 form. Similar results were obtained for the type 3a E1 protein (lanes 10 to 15), which shows a faster mobility on SDS/PAGE because of the presence of only 5 carbohydrates instead of 6. Figure 28 shows a silver stain of an SDS/PAGE gel run in identical conditions as in Figure 26. A complete overview of the purification procedure is given in Figure 29.

The presence of purified E1 protein was further confirmed by means of western blotting as described in example 4. The dimeric E1 protein appeared to be non-aggregated and free of contaminants. The subtype 1b E1 protein purified from vvHCV40-infected cells according to the above scheme was aminoterminally sequenced on an 477 Perkins-Elmer sequencer and appeared to contain a tyrosine as first residue. This confirmed that the E1 protein had been cleaved by the signal peptidase at the correct position (between A191 and Y192) from its signal sequence. This confirms the finding of Hijikata et al. (1991) that the aminotermminus of the mature E1 protein starts at amino acid position 192.

5.5. Purification of the E2 protein

The E2 protein (amino acids 384 to 673) was purified from RK13 cells infected with vvHCV44 as indicated in Examples 5.1 to 5.4. Figure 30 shows the OD₂₈₀ profile (continuous line) of the lentil lectin chromatography. The dotted line represents the E2

reactivity as detected by ELISA (see example 6). Figure 31 shows the same profiles obtained from gelfiltration chromatography of the lentil-lectin E2 pool (see Figure 30), part of which was reduced and blocked according to the methods as set out in example 5.3., and part of which was immediately applied to the column. Both parts of the E2 pool were run on separate gelfiltration columns. It could be demonstrated that E2 forms covalently-linked aggregates with contaminating proteins if no reduction has been performed. After reduction and blocking, the majority of contaminating proteins segregated into the V_0 fraction. Other contaminating proteins copurified with the E2 protein, were not covalently linked to the E2 protein any more because these contaminants could be removed in a subsequent step. Figure 32 shows an additional Ni^{2+} -IMAC purification step carried out for the E2 protein purification. This affinity purification step employs the 6 histidine residues added to the E2 protein as expressed from vvHCV44. Contaminating proteins either run through the column or can be removed by a 30 mM imidazole wash. Figure 33 shows a silver-stained SDS/PAGE of 0.5 μg of purified E2 protein and a 30 mM imidazole wash. The pure E2 protein could be easily recovered by a 200 mM imidazole elution step. Figure 34 shows an additional desalting step intended to remove imidazole and to be able to switch to the desired buffer, e.g. PBS, carbonate buffer, saline.

Starting from about 50,000 cm^2 of RK13 cells infected with vvHCV11A (or vvHCV40) for the production of E1 or vvHCV41, vvHCV42, vvHCV43, or vvHCV44 for production of E2 protein, the procedures described in examples 5.1 to 5.5 allow the purification of approximately 1.3 mg of E1 protein and 0.6 mg of E2 protein.

It should also be remarked that secreted E2 protein (constituting approximately 30-40%, 60-70% being in the intracellular form) is characterized by aggregate formation (contrary to expectations). The same problem is thus posed to purify secreted E2. The secreted E2 can be purified as disclosed above.

Example 6: ELISA for the detection of anti-E1 or anti-E2 antibodies or for the detection of E1 or E2 proteins

Maxisorb microwell plates (Nunc, Roskilde, Denmark) were coated with 1 volume (e.g. 50 μl or 100 μl or 200 μl) per well of a 5 $\mu\text{g}/\text{ml}$ solution of Streptavidin (Boehringer

Mannheim) in PBS for 16 hours at 4°C or for 1 hour at 37°C. Alternatively, the wells were coated with 1 volume of 5 µg/ml of *Galanthus nivalis* agglutinin (GNA) in 50 mM sodium carbonate buffer pH 9.6 for 16 hours at 4°C or for 1 hour at 37°C. In the case of coating with GNA, the plates were washed 2 times with 400 µl of Washing Solution of the Innatest HCV Ab III kit (Innogenetics, Zwijndrecht, Belgium). Unbound coating surfaces were blocked with 1.5 to 2 volumes of blocking solution (0.1% casein and 0.1% NaN₃ in PBS) for 1 hour at 37°C or for 16 hours at 4°C. Blocking solution was aspirated. Purified E1 or E2 was diluted to 100-1000 ng/ml (concentration measured at A = 280 nm) or column fractions to be screened for E1 or E2 (see example 5), or E1 or E2 in non-purified cell lysates (example 5.1.) were diluted 20 times in blocking solution, and 1 volume of the E1 or E2 solution was added to each well and incubated for 1 hour at 37°C on the Streptavidin- or GNA-coated plates. The microwells were washed 3 times with 1 volume of Washing Solution of the Innatest HCV Ab III kit (Innogenetics, Zwijndrecht, Belgium). Serum samples were diluted 20 times or monoclonal anti-E1 or anti-E2 antibodies were diluted to a concentration of 20 ng/ml in Sample Diluent of the Innatest HCV Ab III kit and 1 volume of the solution was left to react with the E1 or E2 protein for 1 hour at 37°C. The microwells were washed 5 times with 400 µl of Washing Solution of the Innatest HCV Ab III kit (Innogenetics, Zwijndrecht, Belgium). The bound antibodies were detected by incubating each well for 1 hour at 37°C with a goat anti-human or anti-mouse IgG, peroxidase-conjugated secondary antibody (DAKO, Glostrup, Denmark) diluted 1/80,000 in 1 volume of Conjugate Diluent of the Innatest HCV Ab III kit (Innogenetics, Zwijndrecht, Belgium), and color development was obtained by addition of substrate of the Innatest HCV Ab III kit (Innogenetics, Zwijndrecht, Belgium) diluted 100 times in 1 volume of Substrate Solution of the Innatest HCV Ab III kit (Innogenetics, Zwijndrecht, Belgium) for 30 min at 24°C after washing of the plates 3 times with 400 µl of Washing Solution of the Innatest HCV Ab III kit (Innogenetics, Zwijndrecht, Belgium).

Example 7: Follow up of patient groups with different clinical profiles

7.1. Monitoring of anti-E1 and anti-E2 antibodies

5 The current hepatitis C virus (HCV) diagnostic assays have been developed for screening and confirmation of the presence of HCV antibodies. Such assays do not seem to provide information useful for monitoring of treatment or for prognosis of the outcome of disease. However, as is the case for hepatitis B, detection and quantification of anti-envelope antibodies may prove more useful in a clinical setting. To investigate the possibility of the use of anti-E1 antibody titer and anti-E2 antibody titer as prognostic markers for outcome of hepatitis C disease, a series of IFN- α treated patients with long-term sustained response (defined as patients with normal transaminase levels and negative HCV-RNA test (PCR in the 5' non-coding region) in the blood for a period of at least 1 year after treatment) was compared with patients showing no response or showing biochemical response with relapse at the end of treatment.

10 A group of 8 IFN- α treated patients with long-term sustained response (LTR, follow up 1 to 3.5 years, 3 type 3a and 5 type 1b) was compared with 9 patients showing non-complete responses to treatment (NR, follow up 1 to 4 years, 6 type 1b and 3 type 3a). Type 1b (vvHCV-39, see example 2.5.) and 3a E1 (vvHCV-62, see example 2.5.) proteins were expressed by the vaccinia virus system (see examples 3 and 4) and purified to homogeneity (example 5). The samples derived from patients infected with a type 1b hepatitis C virus were tested for reactivity with purified type 1b E1 protein, while samples of a type 3a infection were tested for reactivity of anti-type 3a E1 antibodies in an ELISA as described in example 6. The genotypes of hepatitis C viruses infecting the different patients were determined by means of the Inno-LiPA genotyping assay (Innogenetics, Zwijndrecht, Belgium). Figure 5 shows the anti-E1 signal-to-noise ratios of these patients followed during the course of interferon treatment and during the follow-up period after treatment. LTR cases consistently showed rapidly declining anti-E1 levels (with complete negativation in 3 cases), while anti-E1 levels of NR cases remained approximately constant. Some of the obtained anti-E1 data are shown in Table 2 as average S/N ratios "

SD (mean anti-E1 titer). The anti-E1 titer could be deduced from the signal to noise ratio as show in Figures 5, 6, 7, and 8.

Already at the end of treatment, marked differences could be observed between the 2 groups. Anti-E1 antibody titers had decreased 6.9 times in LTR but only 1.5 times in NR.
5 At the end of follow up, the anti-E1 titers had declined by a factor of 22.5 in the patients with sustained response and even slightly increased in NR. Therefore, based on these data, decrease of anti-E1 antibody levels during monitoring of IFN- α therapy correlates with long-term, sustained response to treatment. The anti-E1 assay may be very useful for prognosis of long-term response to IFN treatment, or to treatment of the hepatitis C disease
10 in general.

This finding was not expected. On the contrary, the inventors had expected the anti-E1 antibody levels to increase during the course of IFN treatment in patients with long term response. As is the case for hepatitis B, the virus is cleared as a consequence of the seroconversion for anti-HBsAg antibodies. Also in many other virus infections, the virus is
15 eliminated when anti-envelope antibodies are raised. However, in the experiments of the present invention, anti-E1 antibodies clearly decreased in patients with a long-term response to treatment, while the antibody-level remained approximately at the same level in non-responding patients. Although the outcome of these experiments was not expected, this non-obvious finding may be very important and useful for clinical diagnosis of HCV
20 infections. As shown in Figures 9, 10, 11, and 12, anti-E2 levels behaved very differently in the same patients studied and no obvious decline in titers was observed as for anti-E1 antibodies. Figure 35 gives a complete overview of the pilot study.

As can be deduced from Table 2, the anti-E1 titers were on average at least 2 times higher at the start of treatment in long term responders compared with incomplete
25 responders to treatment. Therefore, measuring the titer of anti-E1 antibodies at the start of treatment, or monitoring the patient during the course of infection and measuring the anti-E1 titer, may become a useful marker for clinical diagnosis of hepatitis C. Furthermore, the use of more defined regions of the E1 or E2 proteins may become desirable, as shown in example 7.3.

7.2. Analysis of E1 and E2 antibodies in a larger patient cohort

The pilot study lead the inventors to conclude that, in case infection was completely cleared, antibodies to the HCV envelope proteins changed more rapidly than antibodies to the more conventionally studied HCV antigens, with E1 antibodies changing most vigorously. We therefore included more type 1b and 3a-infected LTR and further supplemented the cohort with a matched series of NR, such that both groups included 14 patients each. Some partial responders (PR) and responders with relapse (RR) were also analyzed.

Figure 36 depicts average E1 antibody (E1Ab) and E2 antibody (E2Ab) levels in the LTR and NR groups and Tables 4 and 5 show the statistical analyses. In this larger cohort, higher E1 antibody levels before IFN- α therapy were associated with LTR ($P < 0.03$). Since much higher E1 antibody levels were observed in type 3a-infected patients compared with type 1b-infected patients (Figure 37), the genotype was taken into account (Table 4). Within the type 1b-infected group, LTR also had higher E1 antibody levels than NR at the initiation of treatment [$P < 0.05$]; the limited number of type 3a-infected NR did not allow statistical analysis.

Of antibody levels monitored in LTR during the 1.5-year follow up period, only E1 antibodies cleared rapidly compared with levels measured at initiation of treatment [$P = 0.0058$, end of therapy; $P = 0.0047$ and $P = 0.0051$ at 6 and 12 months after therapy, respectively]. This clearance remained significant within type 1- or type 3-infected LTR (average P values < 0.05). These data confirmed the initial finding that E1Ab levels decrease rapidly in the early phase of resolvment. This feature seems to be independent of viral genotype. In NR, PR, or RR, no changes in any of the antibodies measured were observed throughout the follow up period. In patients who responded favourably to treatment with normalization of ALT levels and HCV-RNA negative during treatment, there was a marked difference between sustained responders (LTR) and responders with a relapse (RR). In contrast to LTR, RR did not show any decreasing E1 antibody levels, indicating the presence of occult HCV infection that could neither be demonstrated by PCR or other classical techniques for detection of HCV-RNA, nor by raised ALT levels. The minute quantities of viral RNA, still present in the RR group during treatment, seemed

to be capable of anti-E1 B cell stimulation. Anti-E1 monitoring may therefore not only be able to discriminate LTR from NR, but also from RR.

7.3. Monitoring of antibodies of defined regions of the E1 protein

5

Although the molecular biological approach of identifying HCV antigens resulted in unprecedented breakthrough in the development of viral diagnostics, the method of immune screening of λ gt11 libraries predominantly yielded linear epitopes dispersed throughout the core and non-structural regions, and analysis of the envelope regions had to await cloning and expression of the E1/E2 region in mammalian cells. This approach sharply contrasts with many other viral infections of which epitopes to the envelope regions had already been mapped long before the deciphering of the genomic structure. Such epitopes and corresponding antibodies often had neutralizing activity useful for vaccine development and/or allowed the development of diagnostic assays with clinical or prognostic significance (e.g. antibodies to hepatitis B surface antigen).

15

As no HCV vaccines or tests allowing clinical diagnosis and prognosis of hepatitis C disease are available today, the characterization of viral envelope regions exposed to immune surveillance may significantly contribute to new directions in HCV diagnosis and prophylaxis.

20

Several 20-mer peptides (Table 3) that overlapped each other by 8 amino acids, were synthesized according to a previously described method (EP-A-0 489 968) based on the HC-J1 sequence (Okamoto et al., 1990). None of these, except peptide env35 (also referred to as E1-35), was able to detect antibodies in sera of approximately 200 HCV cases. Only 2 sera reacted slightly with the env35 peptide. However, by means of the anti-E1 ELISA as described in example 6, it was possible to discover additional epitopes as follows: The anti-E1 ELISA as described in example 6 was modified by mixing 50 μ g/ml of E1 peptide with the 1/20 diluted human serum in sample diluent. Figure 13 shows the results of reactivity of human sera to the recombinant E1 (expressed from vvHCV-40) protein, in the presence of single or of a mixture of E1 peptides. While only 2% of the sera could be detected by means of E1 peptides coated on strips in a Line Immunoassay format, over half of the sera contained anti-E1 antibodies which could be competed by means of

25

30

the same peptides, when tested on the recombinant E1 protein. Some of the murine monoclonal antibodies obtained from Balb/C mice after injection with purified E1 protein were subsequently competed for reactivity to E1 with the single peptides (Figure 14). Clearly, the region of env53 contained the predominant epitope, as the addition of env53 could substantially compete reactivity of several sera with E1, and antibodies to the env31 region were also detected. This finding was surprising, since the env53 and env31 peptides had not shown any reactivity when coated directly to the solid phase.

Therefore peptides were synthesized using technology described by applicant previously (in WO 93/18054). The following peptides were synthesized:

10 peptide env35A-biotin

NH₂-SNSSEAADMIMHTPGCV-GKbiotin (SEQ ID NO 51)

spanning amino acids 208 to 227 of the HCV polyprotein in the E1 region

peptide biotin-env53 ('epitope A')

biotin-GG-ITGHRMAWDMMMNWSPTTAL-COOH (SEQ ID NO 52)

15 spanning amino acids to 313 of 332 of the HCV polyprotein in the E1 region

peptide 1bE1 ('epitope B')

H₂N-YEVRNVSGIYHVTNDCSNSSIVYEAADMIMHTPGCGK -biotin
(SEQ ID NO 53)

20 spanning amino acids 192 to 228 of the HCV polyprotein in the E1 region

and compared with the reactivities of peptides E1a-BB (biotin-GG-

TPTVATRDGKLPATQLRRHIDLL, SEQ ID NO 54) and E1b-BB (biotin-GG-

TPTLAARDASVPTTTIRRHVDLL, SEQ ID NO 55) which are derived from the same

region of sequences of genotype 1a and 1b respectively and which have been described at

25 the IXth international virology meeting in Glasgow, 1993 ('epitope C'). Reactivity of a

panel of HCV sera was tested on epitopes A, B and C and epitope B was also compared

with env35A (of 47 HCV-positive sera, 8 were positive on epitope B and none reacted with

env35A). Reactivity towards epitopes A, B, and C was tested directly to the biotinylated

peptides (50 µg/ml) bound to streptavidin-coated plates as described in example 6. Clearly,

30 epitopes A and B were most reactive while epitopes C and env35A-biotin were much less

reactive. The same series of patients that had been monitored for their reactivity towards

the complete E1 protein (example 7.1.) was tested for reactivity towards epitopes A, B, and C. Little reactivity was seen to epitope C, while as shown in Figures 15, 16, 17, and 18, epitopes A and B reacted with the majority of sera. However, antibodies to the most reactive epitope (epitope A) did not seem to predict remission of disease, while the anti-
5 1bE1 antibodies (epitope B) were present almost exclusively in long term responders at the start of IFN treatment. Therefore, anti-1bE1 (epitope B) antibodies and anti-env53 (epitope A) antibodies could be shown to be useful markers for prognosis of hepatitis C disease. The env53 epitope may be advantageously used for the detection of cross-reactive antibodies (antibodies that cross-react between major genotypes) and antibodies to the
10 env53 region may be very useful for universal E1 antigen detection in serum or liver tissue. Monoclonal antibodies that recognized the env53 region were reacted with a random epitope library. In 4 clones that reacted upon immunoscreening with the monoclonal antibody 5E1A10, the sequence -GWD- was present. Because of its analogy with the universal HCV sequence present in all HCV variants in the env53 region, the sequence
15 AWD is thought to contain the essential sequence of the env53 cross-reactive murine epitope. The env31 clearly also contains a variable region which may contain an epitope in the amino terminal sequence -YQVRNSTGL- (SEQ ID NO 93) and may be useful for diagnosis. Env31 or E1-31 as shown in Table 3, is a part of the peptide 1bE1. Peptides E1-33 and E1-51 also reacted to some extent with the murine antibodies, and peptide E1-55
20 (containing the variable region 6 (V6); spanning amino acid positions 329-336) also reacted with some of the patient sera.

Anti-E2 antibodies clearly followed a different pattern than the anti-E1 antibodies, especially in patients with a long-term response to treatment. Therefore, it is clear that the decrease in anti-envelope antibodies could not be measured as efficiently with an assay
25 employing a recombinant E1/E2 protein as with a single anti-E1 or anti-E2 protein. The anti-E2 response would clearly blur the anti-E1 response in an assay measuring both kinds of antibodies at the same time. Therefore, the ability to test anti-envelope antibodies to the single E1 and E2 proteins, was shown to be useful.

7.4. Mapping of anti-E2 antibodies

Of the 24 anti-E2 Mabs only three could be competed for reactivity to recombinant E2 by peptides, two of which reacted with the HVRI region (peptides E2-67 and E2-69, designated as epitope A) and one which recognized an epitope competed by peptide E2-13B (epitope C). The majority of murine antibodies recognized conformational anti-E2 epitopes (Figure 19). A human response to HVRI (epitope A), and to a lesser extent HVRII (epitope B) and a third linear epitope region (competed by peptides E2-23, E2-25 or E2-27, designated epitope E) and a fourth linear epitope region (competed by peptide E2-17B, epitope D) could also frequently be observed, but the majority of sera reacted with conformational epitopes (Figure 20). These conformational epitopes could be grouped according to their relative positions as follows: the IgG antibodies in the supernatant of hybridomas 15C8C1, 12D11F1, 9G3E6, 8G10D1H9, 10D3C4, 4H6B2, 17F2C2, 5H6A7, 15B7A2 recognizing conformational epitopes were purified by means of protein A affinity chromatography and 1 mg/ml of the resulting IgG's were biotinylated in borate buffer in the presence of biotin. Biotinylated antibodies were separated from free biotin by means of gelfiltration chromatography. Pooled biotinylated antibody fractions were diluted 100 to 10,000 times. E2 protein bound to the solid phase was detected by the biotinylated IgG in the presence of 100 times the amount of non-biotinylated competing antibody and subsequently detected by alkaline phosphatase labeled streptavidin.

Percentages of competition are given in Table 6. Based on these results, 4 conformational anti-E2 epitope regions (epitopes F, G, H and I) could be delineated (Figure 38). Alternatively, these Mabs may recognize mutant linear epitopes not represented by the peptides used in this study. Mabs 4H6B2 and 10D3C4 competed reactivity of 16A6E7, but unlike 16A6E7, they did not recognize peptide E2-13B. These Mabs may recognize variants of the same linear epitope (epitope C) or recognize a conformational epitope which is sterically hindered or changes conformation after binding of 16A6E7 to the E2-13B region (epitope H).

Example 8: E1 glycosylation mutants

8.1. Introduction

5 The E1 protein encoded by vvHCV10A, and the E2 protein encoded by vvHCV41 to 44 expressed from mammalian cells contain 6 and 11 carbohydrate moieties, respectively. This could be shown by incubating the lysate of vvHCV10A-infected or vvHCV44-infected RK13 cells with decreasing concentrations of glycosidases (PNGase F or Endoglycosidase H, (Boehringer Mannheim Biochemica) according to the
10 manufacturer's instructions), such that the proteins in the lysate (including E1) are partially deglycosylated (Fig. 39 and 40, respectively).

 Mutants devoid of some of their glycosylation sites could allow the selection of envelope proteins with improved immunological reactivity. For HIV for example, gp120 proteins lacking certain selected sugar-addition motifs, have been found to be particularly
15 useful for diagnostic or vaccine purpose. The addition of a new oligosaccharide side chain in the hemagglutinin protein of an escape mutant of the A/Hong Kong/3/68 (H3N2) influenza virus prevents reactivity with a neutralizing monoclonal antibody (Skehel et al, 1984). When novel glycosylation sites were introduced into the influenza hemagglutinin protein by site-specific mutagenesis, dramatic antigenic changes were observed, suggesting
20 that the carbohydrates serve as a modulator of antigenicity (Gallagher et al., 1988). In another analysis, the 8 carbohydrate-addition motifs of the surface protein gp70 of the Friend Murine Leukemia Virus were deleted. Although seven of the mutations did not affect virus infectivity, mutation of the fourth glycosylation signal with respect to the amino terminus resulted in a non-infectious phenotype (Kayman et al., 1991). Furthermore,
25 it is known in the art that addition of N-linked carbohydrate chains is important for stabilization of folding intermediates and thus for efficient folding, prevention of misfolding and degradation in the endoplasmic reticulum, oligomerization, biological activity, and transport of glycoproteins (see reviews by Rose et al., 1988; Doms et al., 1993; Helenius, 1994).

30 After alignment of the different envelope protein sequences of HCV genotypes, it may be inferred that not all 6 glycosylation sites on the HCV subtype 1b E1 protein are

required for proper folding and reactivity, since some are absent in certain (sub)types. The fourth carbohydrate motif (on Asn251), present in types 1b, 6a, 7, 8, and 9, is absent in all other types known today. This sugar-addition motif may be mutated to yield a type 1b E1 protein with improved reactivity. Also the type 2b sequences show an extra glycosylation site in the V5 region (on Asn299). The isolate S83, belonging to genotype 2c, even lacks the first carbohydrate motif in the V1 region (on Asn), while it is present on all other isolates (Stuyver et al., 1994). However, even among the completely conserved sugar-addition motifs, the presence of the carbohydrate may not be required for folding, but may have a role in evasion of immune surveillance. Therefore, identification of the carbohydrate addition motifs which are not required for proper folding (and reactivity) is not obvious, and each mutant has to be analyzed and tested for reactivity. Mutagenesis of a glycosylation motif (NXS or NXT sequences) can be achieved by either mutating the codons for N, S, or T, in such a way that these codons encode amino acids different from N in the case of N, and/or amino acids different from S or T in the case of S and in the case of T. Alternatively, the X position may be mutated into P, since it is known that NPS or NPT are not frequently modified with carbohydrates. After establishing which carbohydrate-addition motifs are required for folding and/or reactivity and which are not, combinations of such mutations may be made.

8.2. Mutagenesis of the E1 protein

All mutations were performed on the E1 sequence of clone HCC110A (SEQ ID NO. 5). The first round of PCR was performed using sense primer 'GPT' (see Table 7) targeting the GPT sequence located upstream of the vaccinia 11K late promoter, and an antisense primer (designated GLY#, with # representing the number of the glycosylation site, see Fig. 41) containing the desired base change to obtain the mutagenesis. The six GLY# primers (each specific for a given glycosylation site) were designed such that:

- Modification of the codon encoding for the N-glycosylated Asn (AAC or AAT) to a Gln codon (CAA or CAG). Glutamine was chosen because it is very similar to asparagine (both amino acids are neutral and contain non-polar residues, glutamine has a longer side chain (one more -CH₂- group).

- The introduction of silent mutations in one or several of the codons downstream of the glycosylation site, in order to create a new unique or rare (e.g. a second *Sma*I site for E1Gly5) restriction enzyme site. Without modifying the amino acid sequence, this mutation will provide a way to distinguish the mutated sequences from the original E1
5 sequence (pvHCV-10A) or from each other (Figure 41) . This additional restriction site may also be useful for the construction of new hybrid (double, triple, etc.) glycosylation mutants.

- 18 nucleotides extend 5' of the first mismatched nucleotide and 12 to 16 nucleotides extend to the 3' end. Table 7 depicts the sequences of the six GLY# primers overlapping
10 the sequence of N-linked glycosylation sites.

For site-directed mutagenesis, the 'mispriming' or 'overlap extension' (Horton, 1993) was used. The concept is illustrated in Figures 42 and 43. First, two separate fragments were amplified from the target gene for each mutated site. The PCR product obtained from the 5' end (product GLY#) was amplified with the 5' sense GPT primer (see
15 Table 7) and with the respective 3' antisense GLY# primers. The second fragment (product OVR#) was amplified with the 3' antisense TK_R primer and the respective 5' sense primers (OVR# primers, see Table 7, Figure 43).

The OVR# primers target part of the GLY# primer sequence. Therefore, the two groups of PCR products share an overlap region of identical sequence. When these
20 intermediate products are mixed (GLY-1 with OVR-1, GLY-2 with OVR-2, etc.), melted at high temperature, and reannealed, the top sense strand of product GLY# can anneal to the antisense strand of product OVR# (and vice versa) in such a way that the two strands act as primers for one another (see Fig. 42.B.). Extension of the annealed overlap by Taq polymerase during two PCR cycles created the full-length mutant molecule E1Gly#, which
25 carries the mutation destroying the glycosylation site number #. Sufficient quantities of the E1GLY# products for cloning were generated in a third PCR by means of a common set of two internal nested primers. These two new primers are respectively overlapping the 3' end of the vaccinia 11K promoter (sense GPT-2 primer) and the 5' end of the vaccinia thymidine kinase locus (antisense TK_R-2 primer, see Table 7). All PCR conditions were
30 performed as described in Stuyver et al. (1993).

Each of these PCR products was cloned by EcoRI/BamHI cleavage into the EcoRI/BamHI-cut vaccinia vector containing the original E1 sequence (pvHCV-10A).

The selected clones were analyzed for length of insert by EcoRI/BamHI cleavage and for the presence of each new restriction site. The sequences overlapping the mutated sites were confirmed by double-stranded sequencing.

8.3. Analysis of E1 glycosylation mutants

Starting from the 6 plasmids containing the mutant E1 sequences as described in example 8.2, recombinant vaccinia viruses were generated by recombination with wt vaccinia virus as described in example 2.5. Briefly, 175 cm²-flasks of subconfluent RK13 cells were infected with the 6 recombinant vaccinia viruses carrying the mutant E1 sequences, as well as with the vvHCV-10A (carrying the non-mutated E1 sequence) and wt vaccinia viruses. Cells were lysed after 24 hours of infection and analyzed on western blot as described in example 4 (see Figure 44A). All mutants showed a faster mobility (corresponding to a smaller molecular weight of approximately 2 to 3 kDa) on SDS-PAGE than the original E1 protein; confirming that one carbohydrate moiety was not added. Recombinant viruses were also analyzed by PCR and restriction enzyme analysis to confirm the identity of the different mutants. Figure 44B shows that all mutants (as shown in Figure 41) contained the expected additional restriction sites. Another part of the cell lysate was used to test the reactivity of the different mutant by ELISA. The lysates were diluted 20 times and added to microwell plates coated with the lectin GNA as described in example 6. Captured (mutant) E1 glycoproteins were left to react with 20-times diluted sera of 24 HCV-infected patients as described in example 6. Signal to noise (S/N) values (OD of GLY#/OD of wt) for the six mutants and E1 are shown in Table 8. The table also shows the ratios between S/N values of GLY# and E1 proteins. It should be understood that the approach to use cell lysates of the different mutants for comparison of reactivity with patient sera may result in observations that are the consequence of different expression levels rather than reactivity levels. Such difficulties can be overcome by purification of the different mutants as described in example 5, and by testing identical quantities of all the different E1 proteins. However, the results shown in table 5 already

indicate that removal of the 1st (GLY1), 3rd (GLY3), and 6th (GLY6) glycosylation motifs reduces reactivity of some sera, while removal of the 2nd and 5th site does not. Removal of GLY4 seems to improve the reactivity of certain sera. These data indicate that different patients react differently to the glycosylation mutants of the present invention. Thus, such mutant E1 proteins may be useful for the diagnosis (screening, confirmation, prognosis, etc.) and prevention of HCV disease.

Example 9: Expression of HCV E2 protein in glycosylation-deficient yeasts

The E2 sequence corresponding to clone HCCL41 was provided with the α -mating factor pre/pro signal sequence, inserted in a yeast expression vector and *S. cerevisiae* cells transformed with this construct secreted E2 protein into the growth medium. It was observed that most glycosylation sites were modified with high-mannose type glycosylations upon expression of such a construct in *S. cerevisiae* strains (Figure 45). This resulted in a too high level of heterogeneity and in shielding of reactivity, which is not desirable for either vaccine or diagnostic purposes. To overcome this problem, *S. cerevisiae* mutants with modified glycosylation pathways were generated by means of selection of vanadate-resistant clones. Such clones were analyzed for modified glycosylation pathways by analysis of the molecular weight and heterogeneity of the glycoprotein invertase. This allowed us to identify different glycosylation deficient *S. cerevisiae* mutants. The E2 protein was subsequently expressed in some of the selected mutants and left to react with a monoclonal antibody as described in example 7, on western blot as described in example 4 (Figure 46).

Example 10. General utility

The present results show that not only a good expression system but also a good purification protocol are required to reach a high reactivity of the HCV envelope proteins with human patient sera. This can be obtained using the proper HCV envelope protein expression system and/or purification protocols of the present invention which guarantee the conservation of the natural folding of the protein and the purification protocols of the

present invention which guarantee the elimination of contaminating proteins and which preserve the conformation, and thus the reactivity of the HCV envelope proteins. The amounts of purified HCV envelope protein needed for diagnostic screening assays are in the range of grams per year. For vaccine purposes, even higher amounts of envelope
5 protein would be needed. Therefore, the vaccinia virus system may be used for selecting the best expression constructs and for limited upscaling, and large-scale expression and purification of single or specific oligomeric envelope proteins containing high-mannose carbohydrates may be achieved when expressed from several yeast strains. In the case of hepatitis B for example, manufacturing of HBsAg from mammalian cells was much more
10 costly compared with yeast-derived hepatitis B vaccines.

The purification method disclosed in the present invention may also be used for 'viral' envelope proteins' in general. Examples are those derived from Flaviviruses, the newly discovered GB-A, GB-B and GB-C Hepatitis viruses, Pestiviruses (such as Bovine viral Diarrhoea Virus (BVDV), Hog Cholera Virus (HCV), Border Disease Virus (BDV)),
15 but also less related viruses such as Hepatitis B Virus (mainly for the purification of HBsAg).

The envelope protein purification method of the present invention may be used for intra- as well as extracellularly expressed proteins in lower or higher eukaryotic cells or in prokaryotes as set out in the detailed description section.
20

Table 1: Recombinant vaccinia plasmids and viruses

| Plasmid name | Name | cDNA subclone construction | Length (nt/aa) | Vector used for insertion |
|--------------|---------------|----------------------------|----------------|---------------------------|
| pvHCV-13A | E1s | EcoR I - Hind III | 472/157 | pgptATA-18 |
| pvHCV-12A | E1s | EcoR I - Hind III | 472/158 | pgptATA-18 |
| pvHCV-9A | E1 | EcoR I - Hind III | 631/211 | pgptATA-18 |
| pvHCV-11A | E1s | EcoR I - Hind III | 625/207 | pgptATA-18 |
| pvHCV-17A | E1s | EcoR I - Hind III | 625/208 | pgptATA-18 |
| pvHCV-10A | E1 | EcoR I - Hind III | 783/262 | pgptATA-18 |
| pvHCV-18A | COREs | Acc I (Kl) - EcoR I (Kl) | 403/130 | pgptATA-18 |
| pvHCV-34 | CORE | Acc I (Kl) - Fsp I | 595/197 | pgptATA-18 |
| pvHCV-33 | CORE-E1 | Acc I (Kl) | 1150/380 | pgptATA-18 |
| pvHCV-35 | CORE-E1b.his | EcoR I - BamH I (Kl) | 1032/352 | pMS-66 |
| pvHCV-36 | CORE-E1n.his | EcoR I - Nco I (Kl) | 1106/376 | pMS-66 |
| pvHCV-37 | E1Δ | Xma I - BamH I | 711/239 | pvHCV-10A |
| pvHCV-38 | E1Δs | EcoR I - BstE II | 553/183 | pvHCV-11A |
| pvHCV-39 | E1Δb | EcoR I - BamH I | 960/313 | pgsATA-18 |
| pvHCV-40 | E1Δb.his | EcoR I - BamH I (Kl) | 960/323 | pMS-66 |
| pvHCV-41 | E2bs | BamH I (Kl)-AlwN I (T4) | 1005/331 | pgsATA-18 |
| pvHCV-42 | E2bs.his | BamH I (Kl)-AlwN I (T4) | 1005/341 | pMS-66 |
| pvHCV-43 | E2ns | Nco I (Kl) - AlwN I (T4) | 932/314 | pgsATA-18 |
| pvHCV-44 | E2ns.his | Nco I (Kl) - AlwN I (T4) | 932/321 | pMS-66 |
| pvHCV-62 | E1s (type 3a) | EcoR I - Hind III | 625/207 | pgsATA-18 |
| pvHCV-63 | E1s (type 5) | EcoR I - Hind III | 625/207 | pgsATA-18 |
| pvHCV-64 | E2 | BamH I - Hind III | 1410/463 | pgsATA-18 |
| pvHCV-65 | E1-E2 | BamH I - Hind III | 2072/691 | pvHCV-10A |
| pvHCV-66 | CORE-E1-E2 | BamH I - Hind III | 2427/809 | pvHCV-33 |

nt: nucleotide aa: aminoacid Kl: Klenow DNA Pol filling T4: T4 DNA Pol filling

Position: aminoacid position in the HCV polyprotein sequence

Table 1 - continued: Recombinant vaccinia plasmids and viruses

| Plasmid Name | HCV cDNA subclone | | | Vector used for insertion |
|-----------------|-------------------|----------------|-------------------|---------------------------------|
| | Name | Construction | Length (nt/aa) | |
| pvHCV-81 | E1*-GLY 1 | EcoRI - BamH I | 783/262 | pvHCV-10A |
| pvHCV-82 | E1*-GLY 2 | EcoRI - BamH I | 783/262 | pvHCV-10A |
| pvHCV-83 | E1*-GLY 3 | EcoRI - BamH I | 783/262 | pvHCV-10A |
| pvHCV-84 | E1*-GLY 4 | EcoRI - BamH I | 783/262 | pvHCV-10A |
| pvHCV-85 | E1*-GLY 5 | EcoRI - BamH I | 783/262 | pvHCV-10A |
| pvHCV-86 | E1*-GLY 6 | EcoRI - BamH I | 783/262 | pvHCV-10A |

nt: nucleotide aa: aminoacid Kl: Klenow DNA Pol filling T4: T4 DNA Pol filling
Position: aminoacid position in the HCV polyprotein sequence

Table 2 : Summary of anti-E1 tests

S/N \pm SD (mean anti-E1 titer)

| | Start of treatment | End of treatment | Follow-up |
|-----|--------------------------|--------------------------|--------------------------|
| LTR | 6.94 \pm 2.29 (1:3946) | 4.48 \pm 2.69 (1:568) | 2.99 \pm 2.69 (1:175) |
| NR | 5.77 \pm 3.77 (1:1607) | 5.29 \pm 3.99 (1:1060) | 6.08 \pm 3.73 (1:1978) |

LTR : Long-term, sustained response for more than 1 year

NR : No response, response with relapse, or partial response

Table 3

Synthetic peptides for competition studies

| PROTEIN | PEPTIDE | AMINO ACID SEQUENCE | POSITION | SEQ ID NO |
|---------|---------|----------------------|----------|-----------|
| E1 | E1-31 | LLSCLTVPASAYQVRNSTGL | 181-200 | 56 |
| | E1-33 | QVRNSTGLYHVTNDCPNSSI | 193-212 | 57 |
| | E1-35 | NDCPNSSIVYEAHDAILHTP | 205-224 | 58 |
| | E1-35A | SNSSIVYEAADMIMHTPGCV | 208-227 | 59 |
| | E1-37 | HDAILHTPGCVPCVREGNVS | 217-236 | 60 |
| | E1-39 | CVREGNVSRCWVAMTPTVAT | 229-248 | 61 |
| | E1-41 | AMTPTVATRDGKLPATQLRR | 241-260 | 62 |
| | E1-43 | LPATQLRRHIDLLVGSATLC | 253-272 | 63 |
| | E1-45 | LVGSATLCSALYVGDLCSGV | 265-284 | 64 |
| | E1-49 | QLFTFSPRRHWTTQGCNCSI | 289-308 | 65 |
| | E1-51 | TQGCNCSIYPGHITGHRMAW | 301-320 | 66 |
| | E1-53 | ITGHRMAWDMMMNWSPTAAL | 313-332 | 67 |
| | E1-55 | NWSPTAALVMAQLLRIPQAI | 325-344 | 68 |
| | E1-57 | LLRIPQAILDMIAGAHWGVL | 337-356 | 69 |
| | E1-59 | AGAHWGVLAGIAYFSMVGNM | 349-368 | 70 |
| | E1-63 | VVLLLFAGVDAETIVSGGQA | 373-392 | 71 |

| | | | | |
|----|---------|-----------------------|---------|----|
| E2 | E2-67 | SGLVSLFTPGAKQNIQLINT | 397-416 | 72 |
| | E2-69 | QNIQLINTNGSWHINSTALN | 409-428 | 73 |
| | E2-\$3B | LNCNESLNTGWWLAGLIYQHK | 427-446 | 74 |
| | E2-\$1B | AGLIYQHKFNSSGCPERLAS | 439-458 | 75 |
| | E2-1B | GCPERLASCRPLTDFDQGWG | 451-470 | 76 |
| | E2-3B | TDFDQGWGPISYANGSGPDQ | 463-482 | 77 |
| | E2-5B | ANGSGPDQRPYCWHYPPKPC | 475-494 | 78 |
| | E2-7B | WHYPPKPCGIVPAKSVCGPV | 487-506 | 79 |
| | E2-9B | AKSVCGPVYCFTPSPVVVGT | 499-518 | 80 |
| | E2-11B | PSPVVVGTTDRSGAPTYSWG | 511-530 | 81 |
| | E2-13B | GAPTYSWGENDTDVFVLNNT | 523-542 | 82 |
| | E2-17B | GNWFGCTWMNSTGFTKVCGA | 547-566 | 83 |
| | E2-19B | GFTKVCGAPPVCIGGAGNNT | 559-578 | 84 |
| | E2-21 | IGGAGNNTLHCPTDCFRKHP | 571-590 | 85 |
| | E2-23 | TDCFRKHPDATYSRCGSGPW | 583-602 | 86 |
| | E2-25 | SRCGSGPWITPRCLVDYPYR | 595-614 | 87 |
| | E2-27 | CLVDYPYRLWHYPCTINYTI | 607-626 | 88 |
| | E2-29 | PCTINYTIFKIRMYVGGVEH | 619-638 | 89 |
| | E2-31 | MYVGGVEHRLEAACNWTPE | 631-650 | 90 |
| | E2-33 | ACNWTPEGERCDLEDRDRSEL | 643-662 | 91 |
| | E2-35 | EDRDRSELSPLLLTTTQWQV | 655-674 | 92 |

Table 4. Change of Envelope Antibody levels over time (complete study, 28 patients)

| Wilcoxon Signed Rank test (P values) | E1Ab NR All | E1Ab NR type 1b | E1Ab NR type 3a | E1Ab LTR All | E1Ab LTR type 1b | E1Ab LTR type 3a | E2Ab NR All | E1Ab LTR All |
|--------------------------------------|----------------|--------------------|--------------------|-----------------|---------------------|---------------------|----------------|-----------------|
| End of therapy* | 0.1167 | 0.2604 | 0.285 | 0.0058** | 0.043** | 0.0499** | 0.0186** | 0.0640 |
| 6 months follow up* | 0.86 | 0.7213 | 0.5930 | 0.0047** | 0.043** | 0.063 | 0.04326 | 0.0464** |
| 12 months follow up* | 0.7989 | 0.3105 | 1 | 0.0051** | 0.0679 | 0.0277** | 0.0869 | 0.0038** |

* Data were compared with values obtained at initiation of therapy

** P values < 0.05

Table 5. Difference between LTR and NR (complete study)

| Mann-Whitney | E1Ab S/N | E1Ab titers | E1Ab S/N | E1Ab S/N | E2Ab S/N |
|-----------------------|----------|-------------|----------|----------|----------|
| U test (P values) | All | All | type 1b | type 3a | All |
| Initiation of therapy | 0.0257* | | 0.05* | 0.68 | 0.1078 |
| End of therapy | 0.1742 | | | | 0.1295 |
| 6 months follow up | 1 | | 0.6099 | 0.425 | 0.3081 |
| 12 months follow up | 0.67 | | 0.23 | 0.4386 | 0.6629 |

* P values < 0.05

Table 7. Primers

| | | |
|-------------------|--------------------|---|
| SEQ ID NO. 96 | GPT | 5'-GTTTAACCACTGCATGATG-3' |
| SEQ ID NO. 97 | TK _R | 5'-GTCCCATCGAGTGGGCTAC-3' |
| 5 SEQ ID NO. 98 | GLY1 | 5'-CGTGACATGGTACATTCGGGACACTTGGCGCACTTCATAAGCGGA-3' |
| SEQ ID NO. 99 | GLY2 | 5'-TGCCTCATACACAATGAGCCTCTGGGACGAGTCGTTCTGTGAC-3' |
| SEQ ID NO. 100 | GLY3 | 5'-TACCCAGCAGCGGGAGGCTCTGTTGCTCCCGAACGCGAGGGCAC-3' |
| SEQ ID NO. 101 | GLY4 | 5'-TGTCGTGGTGGGACGGAGGCCCTGCCTAGCTGCCGAGCGTGGG-3' |
| SEQ ID NO. 102 | GLY5 | 5'-CGTTATGTGGCCCGGGGTAGATTGAGCACTGGCAGTCCTGCACCGTCTC-3' |
| 10 SEQ ID NO. 103 | GLY6 | 5'-CAGGGCCGTTGTAGGCCCTCCACTGCATCATCATATCCCAAGC-3' |
| SEQ ID NO. 104 | OVR1 | 5'-CCGGAATGTACCATGTACGACGAC-3' |
| SEQ ID NO. 105 | OVR2 | 5'-GCTCCATTGTGTATGAGCAGCGG-3' |
| SEQ ID NO. 106 | OVR3 | 5'-GAGCTCCCGCTGCTGGGTAGCGC-3' |
| SEQ ID NO. 107 | OVR4 | 5'-CCCTCCGTCCCCACCAACGACAATACG-3' |
| 15 SEQ ID NO. 108 | OVR5 | 5'-CTACCCGGGCGCACATAACGGGTCACCG-3' |
| SEQ ID NO. 109 | OVR6 | 5'-GGAGGCCCTACAACGGCCCTGGTGG-3' |
| SEQ ID NO. 110 | GPT-2 | 5'-TTCTATCGATTAAATAGAATTC-3' |
| SEQ ID NO. 111 | TK _R -2 | 5'-GCCATACGCTCACAGCCGATCCCC-3' |

nucleotides underlined represent additional restriction site

20 nucleotides in bold represent mutations with respect to the original HCC10A sequence

2019-03-03

Table B. Analysis of E1 glycosylation mutants by ELISA

| SERUM | | | | | | | | | | | | | Average | |
|---------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Sum | S/N |
| SN GLY1 | 1.002462 | 2.120971 | 1.403871 | 1.205597 | 2.120191 | 2.006913 | 1.950345 | 1.865183 | 1.730193 | 2.468162 | 1.220654 | 1.629403 | | |
| SN GLY2 | 2.400795 | 1.76810 | 2.325495 | 2.639308 | 2.459019 | 5.043993 | 2.146302 | 1.595477 | 1.680973 | 2.402212 | 1.467582 | 2.070524 | | |
| SN GLY3 | 1.842718 | 1.715477 | 2.261646 | 2.354748 | 1.591018 | 4.033742 | 1.96692 | 1.402099 | 1.602222 | 2.191550 | 1.464216 | 1.721164 | | |
| SN GLY4 | 2.570154 | 3.824038 | 3.074605 | 1.499307 | 3.15 | 4.711302 | 4.198751 | 3.959542 | 3.710507 | 5.170841 | 4.250784 | 3.955153 | | |
| SN GLY5 | 2.402051 | 1.793761 | 2.409344 | 2.627358 | 1.715311 | 4.964765 | 2.13912 | 1.576336 | 1.708937 | 3.021007 | 1.562092 | 2.07278 | | |
| SN GLY6 | 2.031487 | 1.495737 | 2.131613 | 2.527925 | 2.494833 | 4.784027 | 2.02069 | 1.496409 | 1.704976 | 2.677757 | 1.529608 | 1.744221 | | |
| SN E1 | 2.828205 | 2.227036 | 2.512792 | 2.790881 | 3.131579 | 4.869128 | 2.287753 | 1.954198 | 1.805556 | 2.616022 | 1.55719 | 2.593886 | | |
| | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | Sum | Average |
| SN GLY1 | 5.685561 | 3.233604 | 3.763490 | 1.985105 | 2.317721 | 6.675179 | 1.93476 | 2.47171 | 4.378633 | 1.180748 | 2.150809 | 1.706992 | 59.80534 | 2.495223 |
| SN GLY2 | 7.556682 | 2.567613 | 3.621920 | 3.055649 | 2.933792 | 7.65433 | 2.127712 | 2.921208 | 4.60101 | 1.150781 | 1.061914 | 1.632785 | 69.65243 | 2.902185 |
| SN GLY3 | 7.930538 | 2.783055 | 3.016099 | 2.945628 | 2.515305 | 5.775357 | 1.980185 | 2.557304 | 4.268633 | 0.97767 | 1.336775 | 1.20376 | 62.09872 | 2.597447 |
| SN GLY4 | 8.176816 | 6.561122 | 5.707668 | 5.684498 | 5.604813 | 6.4125 | 3.813321 | 3.002535 | 4.293038 | 2.393011 | 3.68213 | 2.481585 | 102.6978 | 4.279076 |
| SN GLY5 | 8.883408 | 2.940334 | 3.125561 | 3.338912 | 2.654224 | 5.424107 | 2.442804 | 3.126761 | 4.64557 | 1.153656 | 1.817901 | 1.638211 | 69.26511 | 2.880046 |
| SN GLY6 | 8.005561 | 2.499952 | 2.621704 | 2.572385 | 2.363301 | 5.194107 | 1.506716 | 2.685433 | 2.781053 | 1.280743 | 1.475062 | 1.716423 | 61.32181 | 2.555075 |
| SN E1 | 8.825112 | 3.183771 | 3.067265 | 3.280335 | 2.980354 | 7.191964 | 2.771218 | 3.678068 | 5.35443 | 1.167286 | 2.083333 | 1.78252 | 76.54068 | 3.189195 |
| | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | Sum | Average |
| GLY1/E1 | 0.637316 | 0.952374 | 0.55069 | 0.431977 | 0.677036 | 0.508794 | 0.852516 | 0.954961 | 0.950261 | 0.94319 | 0.783082 | 0.620171 | | |
| GLY2/E1 | 0.048876 | 0.793961 | 0.925463 | 0.94569 | 0.785233 | 1.035913 | 0.93017 | 0.818438 | 0.935431 | 0.94856 | 0.942455 | 0.790232 | | |
| GLY3/E1 | 0.580834 | 0.770298 | 0.900053 | 0.84373 | 0.508312 | 0.992733 | 0.859761 | 0.758418 | 0.887385 | 0.837408 | 0.940294 | 0.663547 | | |
| GLY4/E1 | 0.911587 | 1.717097 | 1.541952 | 0.537245 | 1.005802 | 0.967939 | 1.033317 | 2.026172 | 2.05505 | 1.976 | 2.72970 | 1.524798 | | |
| GLY5/E1 | 0.877607 | 0.805447 | 0.958031 | 0.941408 | 0.547746 | 1.019642 | 0.935031 | 0.806641 | 0.946488 | 1.154762 | 1.003148 | 0.799102 | | |
| GLY6/E1 | 0.718296 | 0.671626 | 0.848305 | 0.90578 | 0.798669 | 0.982522 | 0.803264 | 0.765701 | 0.944294 | 1.023286 | 0.902208 | 0.672435 | | |
| | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | Sum | Average |
| GLY1/E1 | 0.644248 | 1.015652 | 1.226988 | 0.605153 | 0.771866 | 0.928144 | 0.690162 | 0.672013 | 0.817759 | 1.010386 | 1.036267 | 0.957628 | 19.36524 | 0.806805 |
| GLY2/E1 | 0.85627 | 0.806469 | 1.180833 | 0.931505 | 0.984377 | 1.064209 | 0.76779 | 0.794245 | 0.874061 | 0.90506 | 0.797719 | 0.915998 | 21.67384 | 0.903077 |
| GLY3/E1 | 0.898033 | 0.867856 | 0.983319 | 0.897988 | 0.843962 | 0.803029 | 0.714554 | 0.895300 | 0.797215 | 0.837550 | 0.641652 | 0.675314 | 19.19921 | 0.799967 |
| GLY4/E1 | 0.92654 | 2.060802 | 1.850833 | 1.732992 | 1.880587 | 0.89162 | 1.376045 | 0.816335 | 0.801773 | 2.050064 | 1.767422 | 1.392178 | 36.38592 | 1.51608 |
| GLY5/E1 | 1.006606 | 0.923538 | 1.019006 | 1.017857 | 0.890574 | 0.75419 | 0.881491 | 0.85612 | 0.867612 | 0.988323 | 0.872593 | 0.919042 | 21.78679 | 0.907783 |
| GLY6/E1 | 0.907134 | 0.785217 | 0.854737 | 0.784184 | 0.79296 | 0.72221 | 0.543702 | 0.724683 | 0.519395 | 1.097197 | 0.708003 | 0.962919 | 19.59691 | 0.816538 |

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30

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Innogenetics N.V.
- (B) STREET: Industriepark Zwijnaarde 7 Bus 4
- (C) CITY: Gent
- (E) COUNTRY: Belgium
- (F) POSTAL CODE (ZIP): 9052
- (G) TELEPHONE: 00-32-09.241.07.11
- (H) TELEFAX: 00-32-09.241.07.99

(ii) TITLE OF INVENTION: Purified hepatitis C virus envelope proteins for diagnostic and therapeutic use.

(iii) NUMBER OF SEQUENCES: 111

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: PCT/EP/95/03031

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GGCATGCAAG CTTAATTAAT T

21

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 68 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CCGGGGAGGC CTGCACGTGA TCGAGGGCAG ACACCATCAC CACCATCACT AATAGTTAAT

50

TAACTGCA

68

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 642 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 1..639

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
 (B) LOCATION: 1..636

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

| | |
|---|-----|
| ATG CCC GGT TGC TCT TTC TCT ATC TTC CTC TTG GCT TTA CTG TCC TGT | 48 |
| Met Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu Leu Ser Cys | |
| 1 5 10 15 | |
| CTG ACC ATT CCA GCT TCC GCT TAT GAG GTG CGC AAC GTG TCC GGG ATG | 96 |
| Leu Thr Ile Pro Ala Ser Ala Tyr Glu Val Arg Asn Val Ser Gly Met | |
| 20 25 30 | |
| TAC CAT GTC ACG AAC GAC TGC TCC AAC TCA AGC ATT GTG TAT GAG GCA | 144 |
| Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val Tyr Glu Ala | |
| 35 40 45 | |
| GCG GAC ATG ATC ATG CAC ACC CCC GGG TGC GTG CCC TGC GTT CGG GAG | 192 |
| Ala Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys Val Arg Glu | |
| 50 55 60 | |
| AAC AAC TCT TCC CGC TGC TGG GTA GCG CTC ACC CCC ACG CTC GCA GCT | 240 |
| Asn Asn Ser Ser Arg Cys Trp Val Ala Leu Thr Pro Thr Leu Ala Ala | |
| 65 70 75 80 | |
| AGG AAC GCC AGC GTC CCC ACC ACG ACA ATA CGA CGC CAC GTC GAT TTG | 288 |
| Arg Asn Ala Ser Val Pro Thr Thr Thr Ile Arg Arg His Val Asp Leu | |
| 85 90 95 | |
| CTC GTT GGG GCG GCT GCT CTC TGT TCC GCT ATG TAC GTG GGG GAT CTC | 336 |
| Leu Val Gly Ala Ala Ala Leu Cys Ser Ala Met Tyr Val Gly Asp Leu | |
| 100 105 110 | |
| TGC GGA TCT GTC TTC CTC GTC TCC CAG CTG TTC ACC ATC TCG CCT CGC | 384 |
| Cys Gly Ser Val Phe Leu Val Ser Gln Leu Phe Thr Ile Ser Pro Arg | |
| 115 120 125 | |
| CGG CAT GAG ACG GTG CAG GAC TGC AAT TGC TCA ATC TAT CCC GGC CAC | 432 |
| Arg His Glu Thr Val Gln Asp Cys Asn Cys Ser Ile Tyr Pro Gly His | |
| 130 135 140 | |
| ATA ACA GGT CAC CGT ATG GCT TGG GAT ATG ATG ATG AAC TGG TCG CCT | 480 |
| Ile Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn Trp Ser Pro | |
| 145 150 155 160 | |
| ACA ACG GCC CTG GTG GTA TCG CAG CTG CTC CGG ATC CCA CAA GCT GTC | 528 |

CTC TTT GCT CTC TAATAG
Leu Phe Ala Leu
210

42

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 212 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

[illegible]

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 795 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..792

(ix) FEATURE:
 (A) NAME/KEY: mat_peptide
 (B) LOCATION: 1..789

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

| | |
|---|-----|
| ATG TTG GGT AAG GTC ATC GAT ACC CTT ACA TGC GGC TTC GCC GAC CTC | 48 |
| Met Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu | |
| 1 5 10 15 | |
| GTG GGG TAC ATT CCG CTC GTC GGC GCC CCC CTA GGG GGC GCT GCC AGG | 96 |
| Val Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg | |
| 20 25 30 | |
| GCC CTG GCG CAT GGC GTC CGG GTT CTG GAG GAC GGC GTG AAC TAT GCA | 144 |
| Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala | |
| 35 40 45 | |
| ACA GGG AAT TTG CCC GGT TGC TCT TTC TCT ATC TTC CTC TTG GCT TTG | 192 |
| Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu | |
| 50 55 60 | |
| CTG TCC TGT CTG ACC GTT CCA GCT TCC GCT TAT GAA GTG CGC AAC GTG | 240 |
| Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr Glu Val Arg Asn Val | |
| 65 70 75 80 | |
| TCC GGG ATG TAC CAT GTC ACG AAC GAC TGC TCC AAC TCA AGC ATT GTG | 288 |
| Ser Gly Met Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val | |
| 85 90 95 | |
| TAT GAG GCA GCG GAC ATG ATC ATG CAC ACC CCC GGG TGC GTG CCC TGC | 336 |
| Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys | |
| 100 105 110 | |
| GTT CGG GAG AAC AAC TCT TCC CGC TGC TGG GTA GCG CTC ACC CCC ACG | 384 |
| Val Arg Glu Asn Asn Ser Ser Arg Cys Trp Val Ala Leu Thr Pro Thr | |
| 115 120 125 | |
| CTC GCA GCT AGG AAC GCC AGC GTC CCC ACC ACG ACA ATA CGA CGC CAC | 432 |
| Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Thr Ile Arg Arg His | |
| 130 135 140 | |
| GTC GAT TTG CTC GTT GGG GCG GCT GCT TTC TGT TCC GCT ATG TAC GTG | 480 |
| Val Asp Leu Leu Val Gly Ala Ala Ala Phe Cys Ser Ala Met Tyr Val | |
| 145 150 155 160 | |
| GGG GAC CTC TGC GGA TCT GTC TTC CTC GTC TCC CAG CTG TTC ACC ATC | 528 |
| Gly Asp Leu Cys Gly Ser Val Phe Leu Val Ser Gln Leu Phe Thr Ile | |
| 165 170 175 | |

| | |
|---|-----|
| TCG CCT CGC CGG CAT GAG ACG GTG CAG GAC TGC AAT TGC TCA ATC TAT | 576 |
| Ser Pro Arg Arg His Glu Thr Val Gln Asp Cys Asn Cys Ser Ile Tyr | |
| 180 185 190 | |
| CCC GGC CAC ATA ACG GGT CAC CGT ATG GCT TGG GAT ATG ATG ATG AAC | 624 |
| Pro Gly His Ile Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn | |
| 195 200 205 | |
| TGG TCG CCT ACA ACG GCC CTG GTG GTA TCG CAG CTG CTC CGG ATC CCA | 672 |
| Trp Ser Pro Thr Thr Ala Leu Val Val Ser Gln Leu Leu Arg Ile Pro | |
| 210 215 220 | |
| CAA GCT GTC GTG GAC ATG GTG GCG GGG GCC CAT TGG GGA GTC CTG GCG | 720 |
| Gln Ala Val Val Asp Met Val Ala Gly Ala His Trp Gly Val Leu Ala | |
| 225 230 235 240 | |
| GGT CTC GCC TAC TAT TCC ATG GTG GGG AAC TGG GCT AAG GTT TTG ATT | 768 |
| Gly Leu Ala Tyr Tyr Ser Met Val Gly Asn Trp Ala Lys Val Leu Ile | |
| 245 250 255 | |
| GTG ATG CTA CTC TTT GCT CCC TAATAG | 795 |
| Val Met Leu Leu Phe Ala Pro | |
| 260 | |

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 263 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

| | |
|---|--|
| Met Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu | |
| 1 5 10 15 | |
| Val Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Arg | |
| 20 25 30 | |
| Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala | |
| 35 40 45 | |
| Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu | |
| 50 55 60 | |
| Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr Glu Val Arg Asn Val | |
| 65 70 75 80 | |
| Ser Gly Met Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val | |
| 85 90 95 | |
| Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys | |
| 100 105 110 | |
| Val Arg Glu Asn Asn Ser Ser Arg Cys Trp Val Ala Leu Thr Pro Thr | |
| 115 120 125 | |
| Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Thr Ile Arg Arg His | |
| 130 135 140 | |
| Val Asp Leu Leu Val Gly Ala Ala Ala Phe Cys Ser Ala Met Tyr Val | |
| 145 150 155 160 | |
| Gly Asp Leu Cys Gly Ser Val Phe Leu Val Ser Gln Leu Phe Thr Ile | |
| 165 170 175 | |

Ser Pro Arg Arg His Glu Thr Val Gln Asp Cys Asn Cys Ser Ile Tyr
180 185 190
Pro Gly His Ile Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn
195 200 205
Trp Ser Pro Thr Thr Ala Leu Val Val Ser Gln Leu Leu Arg Ile Pro
210 215 220
Gln Ala Val Val Asp Met Val Ala Gly Ala His Trp Gly Val Leu Ala
225 230 235 240
Gly Leu Ala Tyr Tyr Ser Met Val Gly Asn Trp Ala Lys Val Leu Ile
245 250 255
Val Met Leu Leu Phe Ala Pro
260

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 633 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..630

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..627

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

| | |
|---|-----|
| ATG TTG GGT AAG GTC ATC GAT ACC CTT ACG TGC GGC TTC GCC GAC CTC | 48 |
| Met Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu | |
| 1 5 10 15 | |
| ATG GGG TAC ATT CCG CTC GTC GGC GCC CCC CTA GGG GGT GCT GCC AGA | 96 |
| Met Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg | |
| 20 25 30 | |
| GCC CTG GCG CAT GGC GTC CGG GTT CTG GAA GAC GGC GTG AAC TAT GCA | 144 |
| Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala | |
| 35 40 45 | |
| ACA GGG AAT TTG CCT GGT TGC TCT TTC TCT ATC TTC CTC TTG GCT TTA | 192 |
| Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu | |
| 50 55 60 | |
| CTG TCC TGT CTG ACC ATT CCA GCT TCC GCT TAT GAG GTG CGC AAC GTG | 240 |
| Leu Ser Cys Leu Thr Ile Pro Ala Ser Ala Tyr Glu Val Arg Asn Val | |
| 65 70 75 80 | |
| TCC GGG ATG TAC CAT GTC ACG AAC GAC TGC TCC AAC TCA AGC ATT GTG | 288 |
| Ser Gly Met Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val | |
| 85 90 95 | |

| | |
|---|-----|
| TAT GAG GCA GCG GAC ATG ATC ATG CAC ACC CCC GGG TGC GTG CCC TGC | 336 |
| Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys | |
| 100 105 110 | |
| GTT CGG GAG AAC AAC TCT TCC CGC TGC TGG GTA GCG CTC ACC CCC ACG | 384 |
| Val Arg Glu Asn Asn Ser Ser Arg Cys Trp Val Ala Leu Thr Pro Thr | |
| 115 120 125 | |
| CTC GCA GCT AGG AAC GCC AGC GTC CCC ACT ACG ACA ATA CGA CGC CAC | 432 |
| Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Ile Arg Arg His | |
| 130 135 140 | |
| GTC GAT TTG CTC GTT GGG GCG GCT GCT TTC TGT TCC GCT ATG TAC GTG | 480 |
| Val Asp Leu Leu Val Gly Ala Ala Phe Cys Ser Ala Met Tyr Val | |
| 145 150 155 160 | |
| GGG GAT CTC TGC GGA TCT GTC TTC CTC GTC TCC CAG CTG TTC ACC ATC | 528 |
| Gly Asp Leu Cys Gly Ser Val Phe Leu Val Ser Gln Leu Phe Thr Ile | |
| 165 170 175 | |
| TCG CCT CGC CGG CAT GAG ACG GTG CAG GAC TGC AAT TGC TCA ATC TAT | 576 |
| Ser Pro Arg Arg His Glu Thr Val Gln Asp Cys Asn Cys Ser Ile Tyr | |
| 180 185 190 | |
| CCC GGC CAC ATA ACA GGT CAC CGT ATG GCT TGG GAT ATG ATG ATG AAC | 624 |
| Pro Gly His Ile Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn | |
| 195 200 205 | |
| TGG TAATAG | 633 |
| Trp | |
| 210 | |

(2) INFORMATION FOR SEQ ID NO: 8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 209 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Leu | Gly | Lys | Val | Ile | Asp | Thr | Leu | Thr | Cys | Gly | Phe | Ala | Asp | Leu |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| Met | Gly | Tyr | Ile | Pro | Leu | Val | Gly | Ala | Pro | Leu | Gly | Gly | Ala | Ala | Arg |
| | | 20 | | | | | | 25 | | | | | 30 | | |
| Ala | Leu | Ala | His | Gly | Val | Arg | Val | Leu | Glu | Asp | Gly | Val | Asn | Tyr | Ala |
| | 35 | | | | | | 40 | | | | | 45 | | | |
| Thr | Gly | Asn | Leu | Pro | Gly | Cys | Ser | Phe | Ser | Ile | Phe | Leu | Leu | Ala | Leu |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Leu | Ser | Cys | Leu | Thr | Ile | Pro | Ala | Ser | Ala | Tyr | Glu | Val | Arg | Asn | Val |
| 65 | | | | 70 | | | | | | 75 | | | | 80 | |
| Ser | Gly | Met | Tyr | His | Val | Thr | Asn | Asp | Cys | Ser | Asn | Ser | Ser | Ile | Val |
| | | | 85 | | | | | | 90 | | | | | 95 | |
| Tyr | Glu | Ala | Ala | Asp | Met | Ile | Met | His | Thr | Pro | Gly | Cys | Val | Pro | Cys |
| | | 100 | | | | | 105 | | | | | | 110 | | |
| Val | Arg | Glu | Asn | Asn | Ser | Ser | Arg | Cys | Trp | Val | Ala | Leu | Thr | Pro | Thr |
| | 115 | | | | | | 120 | | | | | 125 | | | |
| Leu | Ala | Ala | Arg | Asn | Ala | Ser | Val | Pro | Thr | Thr | Thr | Ile | Arg | Arg | His |

| | | | | |
|---|-----|-----|-----|-----|
| 130 | | 135 | | 140 |
| Val Asp Leu Leu Val Gly Ala Ala Ala Phe Cys Ser Ala Met Tyr Val | | | | |
| 145 | | 150 | | 155 |
| Gly Asp Leu Cys Gly Ser Val Phe Leu Val Ser Gln Leu Phe Thr Ile | | | | |
| | 165 | | 170 | 175 |
| Ser Pro Arg Arg His Glu Thr Val Gln Asp Cys Asn Cys Ser Ile Tyr | | | | |
| | 180 | | 185 | 190 |
| Pro Gly His Ile Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn | | | | |
| | 195 | | 200 | 205 |

Trp

(2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 483 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..480

- (ix) FEATURE:
 (A) NAME/KEY: mat_peptide
 (B) LOCATION: 1..477

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

| | |
|---|-----|
| ATG CCC GGT TGC TCT TTC TCT ATC TTC CTC TTG GCC CTG CTG TCC TGT | 48 |
| Met Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu Leu Ser Cys | |
| 1 5 10 15 | |
| CTG ACC ATA CCA GCT TCC GCT TAT GAA GTG CGC AAC GTG TCC GGG GTG | 96 |
| Leu Thr Ile Pro Ala Ser Ala Tyr Glu Val Arg Asn Val Ser Gly Val | |
| 20 25 30 | |
| TAC CAT GTC ACG AAC GAC TGC TCC AAC TCA AGC ATA GTG TAT GAG GCA | 144 |
| Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val Tyr Glu Ala | |
| 35 40 45 | |
| GCG GAC ATG ATC ATG CAC ACC CCC GGG TGC GTG CCC TGC GTT CGG GAG | 192 |
| Ala Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys Val Arg Glu | |
| 50 55 60 | |
| GGC AAC TCC TCC CGT TGC TGG GTG GCG CTC ACT CCC ACG CTC GCG GCC | 240 |
| Gly Asn Ser Ser Arg Cys Trp Val Ala Leu Thr Pro Thr Leu Ala Ala | |
| 65 70 75 80 | |
| AGG AAC GCC AGC GTC CCC ACA ACG ACA ATA CGA CGC CAC GTC GAT TTG | 288 |
| Arg Asn Ala Ser Val Pro Thr Thr Thr Ile Arg Arg His Val Asp Leu | |
| 85 90 95 | |
| CTC GTT GGG GCT GCT GCT TTC TGT TCC GCT ATG TAC GTG GGG GAT CTC | 336 |
| Leu Val Gly Ala Ala Ala Phe Cys Ser Ala Met Tyr Val Gly Asp Leu | |

| 100 | 105 | 110 | |
|--|-----|-----|-----|
| TGC GGA TCT GTT TTC CTT GTT TCC CAG CTG TTC ACC TTC TCA CCT CGC | | | 384 |
| Cys Gly Ser Val Phe Leu Val Ser Gln Leu Phe Thr Phe Ser Pro Arg | | | |
| 115 | 120 | 125 | |
| CGG CAT CAA ACA GTA CAG GAC TGC AAC TGC TCA ATC TAT CCC GGC CAT | | | 432 |
| Arg His Gln Thr Val Gln Asp Cys Asn Cys Ser Ile Tyr Pro Gly His | | | |
| 130 | 135 | 140 | |
| GTA TCA GGT CAC CGC ATG GCT TGG GAT ATG ATG ATG AAC TGG TCC TAATAG | | | 483 |
| Val Ser Gly His Arg Met Ala Trp Asp Met Met Met Asn Trp Ser | | | |
| 145 | 150 | 155 | 160 |

(2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 159 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Pro | Gly | Cys | Ser | Phe | Ser | Ile | Phe | Leu | Leu | Ala | Leu | Leu | Ser | Cys |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| Leu | Thr | Ile | Pro | Ala | Ser | Ala | Tyr | Glu | Val | Arg | Asn | Val | Ser | Gly | Val |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| Tyr | His | Val | Thr | Asn | Asp | Cys | Ser | Asn | Ser | Ser | Ile | Val | Tyr | Glu | Ala |
| | | 35 | | | | 40 | | | | | | 45 | | | |
| Ala | Asp | Met | Ile | Met | His | Thr | Pro | Gly | Cys | Val | Pro | Cys | Val | Arg | Glu |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Gly | Asn | Ser | Ser | Arg | Cys | Trp | Val | Ala | Leu | Thr | Pro | Thr | Leu | Ala | Ala |
| 65 | | | | | 70 | | | | | 75 | | | | 80 | |
| Arg | Asn | Ala | Ser | Val | Pro | Thr | Thr | Thr | Ile | Arg | Arg | His | Val | Asp | Leu |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Leu | Val | Gly | Ala | Ala | Ala | Phe | Cys | Ser | Ala | Met | Tyr | Val | Gly | Asp | Leu |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Cys | Gly | Ser | Val | Phe | Leu | Val | Ser | Gln | Leu | Phe | Thr | Phe | Ser | Pro | Arg |
| | | | 115 | | | | 120 | | | | | 125 | | | |
| Arg | His | Gln | Thr | Val | Gln | Asp | Cys | Asn | Cys | Ser | Ile | Tyr | Pro | Gly | His |
| | 130 | | | | | 135 | | | | | 140 | | | | |
| Val | Ser | Gly | His | Arg | Met | Ala | Trp | Asp | Met | Met | Met | Asn | Trp | Ser | |
| 145 | | | | | 150 | | | | 155 | | | | | | |

(2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 480 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..477

(ix) FEATURE:

(A) NAME/KEY: mat_peptide

(B) LOCATION: 1..474

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------|-----|-----|
| ATG | TCC | GGT | TGC | TCT | TTC | TCT | ATC | TTC | CTC | TTG | GCC | CTG | CTG | TCC | TGT | 48 |
| Met | Ser | Gly | Cys | Ser | Phe | Ser | Ile | Phe | Leu | Leu | Ala | Leu | Leu | Ser | Cys | |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | | |
| CTG | ACC | ATA | CCA | GCT | TCC | GCT | TAT | GAA | GTG | CGC | AAC | GTG | TCC | GGG | GTG | 96 |
| Leu | Thr | Ile | Pro | Ala | Ser | Ala | Tyr | Glu | Val | Arg | Asn | Val | Ser | Gly | Val | |
| | | | 20 | | | | | 25 | | | | | 30 | | | |
| TAC | CAT | GTC | ACG | AAC | GAC | TGC | TCC | AAC | TCA | AGC | ATA | GTG | TAT | GAG | GCA | 144 |
| Tyr | His | Val | Thr | Asn | Asp | Cys | Ser | Asn | Ser | Ser | Ile | Val | Tyr | Glu | Ala | |
| | | 35 | | | | 40 | | | | | | 45 | | | | |
| GCG | GAC | ATG | ATC | ATG | CAC | ACC | CCC | GGG | TGC | GTG | CCC | TGC | GTT | CGG | GAG | 192 |
| Ala | Asp | Met | Ile | Met | His | Thr | Pro | Gly | Cys | Val | Pro | Cys | Val | Arg | Glu | |
| | 50 | | | | 55 | | | | | | 60 | | | | | |
| GGC | AAC | TCC | TCC | CGT | TGC | TGG | GTG | GCG | CTC | ACT | CCC | ACG | CTC | GCG | GCC | 240 |
| Gly | Asn | Ser | Ser | Arg | Cys | Trp | Val | Ala | Leu | Thr | Pro | Thr | Leu | Ala | Ala | |
| | 65 | | | | 70 | | | | | 75 | | | | | 80 | |
| AGG | AAC | GCC | AGC | GTC | CCC | ACA | ACG | ACA | ATA | CGA | CGC | CAC | GTC | GAT | TTG | 288 |
| Arg | Asn | Ala | Ser | Val | Pro | Thr | Thr | Thr | Ile | Arg | Arg | His | Val | Asp | Leu | |
| | | | | 85 | | | | | 90 | | | | | 95 | | |
| CTC | GTT | GGG | GCT | GCT | GCT | TTC | TGT | TCC | GCT | ATG | TAC | GTG | GGG | GAT | CTC | 336 |
| Leu | Val | Gly | Ala | Ala | Ala | Phe | Cys | Ser | Ala | Met | Tyr | Val | Gly | Asp | Leu | |
| | | | 100 | | | | | 105 | | | | | 110 | | | |
| TGC | GGA | TCT | GTT | TTC | CTT | GTT | TCC | CAG | CTG | TTC | ACC | TTC | TCA | CCT | CGC | 384 |
| Cys | Gly | Ser | Val | Phe | Leu | Val | Ser | Gln | Leu | Phe | Thr | Phe | Ser | Pro | Arg | |
| | | 115 | | | | | 120 | | | | | 125 | | | | |
| CGG | CAT | CAA | ACA | GTA | CAG | GAC | TGC | AAC | TGC | TCA | ATC | TAT | CCC | GGC | CAT | 432 |
| Arg | His | Gln | Thr | Val | Gln | Asp | Cys | Asn | Cys | Ser | Ile | Tyr | Pro | Gly | His | |
| | | 130 | | | | 135 | | | | | 140 | | | | | |
| GTA | TCA | GGT | CAC | CGC | ATG | GCT | TGG | GAT | ATG | ATG | ATG | AAC | TGG | TAATAG | | 480 |
| Val | Ser | Gly | His | Arg | Met | Ala | Trp | Asp | Met | Met | Met | Asn | Trp | | | |
| 145 | | | | | 150 | | | | 155 | | | | | | | |

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 158 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ser | Gly | Cys | Ser | Phe | Ser | Ile | Phe | Leu | Leu | Ala | Leu | Leu | Ser | Cys |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |

Leu Thr Ile Pro Ala Ser Ala Tyr Glu Val Arg Asn Val Ser Gly Val
 20 25 30
 Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val Tyr Glu Ala
 35 40 45
 Ala Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys Val Arg Glu
 50 55 60
 Gly Asn Ser Ser Arg Cys Trp Val Ala Leu Thr Pro Thr Leu Ala Ala
 65 70 75 80
 Arg Asn Ala Ser Val Pro Thr Thr Thr Ile Arg Arg His Val Asp Leu
 85 90 95
 Leu Val Gly Ala Ala Ala Phe Cys Ser Ala Met Tyr Val Gly Asp Leu
 100 105 110
 Cys Gly Ser Val Phe Leu Val Ser Gln Leu Phe Thr Phe Ser Pro Arg
 115 120 125
 Arg His Gln Thr Val Gln Asp Cys Asn Cys Ser Ile Tyr Pro Gly His
 130 135 140
 Val Ser Gly His Arg Met Ala Trp Asp Met Met Met Asn Trp
 145 150 155

(2) INFORMATION FOR SEQ ID NO: 13:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 636 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 1..633

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
 (B) LOCATION: 1..630

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

| | |
|---|-----|
| ATG CTG GGT AAG GCC ATC GAT ACC CTT ACG TGC GGC TTC GCC GAC CTC | 48 |
| Met Leu Gly Lys Ala Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu | |
| 1 5 10 15 | |
| GTG GGG TAC ATT CCG CTC GTC GGC GCC CCC CTA GGG GGC GCT GCC AGG | 96 |
| Val Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg | |
| 20 25 30 | |
| GCC CTG GCG CAT GGC GTC CGG GTT CTG GAA GAC GGC GTG AAC TAT GCA | 144 |
| Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala | |
| 35 40 45 | |
| ACA GGG AAT TTG CCT GGT TGC TCT TTC TCT ATC TTC CTC TTG GCT TTA | 192 |
| Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu | |
| 50 55 60 | |

| | | | | | | | | | | | | | | | | |
|-----|-----|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| CTG | TCC | TGT | CTA | ACC | ATT | CCA | GCT | TCC | GCT | TAC | GAG | GTG | CGC | AAC | GTG | 240 |
| Leu | Ser | Cys | Leu | Thr | Ile | Pro | Ala | Ser | Ala | Tyr | Glu | Val | Arg | Asn | Val | |
| 65 | | | | | 70 | | | | | 75 | | | | | 80 | |
| TCC | GGG | ATG | TAC | CAT | GTC | ACG | AAC | GAC | TGC | TCC | AAC | TCA | AGC | ATT | GTG | 288 |
| Ser | Gly | Met | Tyr | His | Val | Thr | Asn | Asp | Cys | Ser | Asn | Ser | Ser | Ile | Val | |
| | | | | 85 | | | | | 90 | | | | | 95 | | |
| TAT | GAG | GCA | GCG | GAC | ATG | ATC | ATG | CAC | ACC | CCC | GGG | TGC | GTG | CCC | TGC | 336 |
| Tyr | Glu | Ala | Ala | Asp | Met | Ile | Met | His | Thr | Pro | Gly | Cys | Val | Pro | Cys | |
| | | | 100 | | | | | 105 | | | | | 110 | | | |
| GTT | CGG | GAG | AAC | AAC | TCT | TCC | CGC | TGC | TGG | GTA | GCG | CTC | ACC | CCC | ACG | 384 |
| Val | Arg | Glu | Asn | Asn | Ser | Ser | Arg | Cys | Trp | Val | Ala | Leu | Thr | Pro | Thr | |
| | | | 115 | | | | 120 | | | | | 125 | | | | |
| CTC | CGC | GCT | AGG | AAC | GCC | AGC | ATC | CCC | ACT | ACA | ACA | ATA | CGA | CGC | CAC | 432 |
| Leu | Ala | Ala | Arg | Asn | Ala | Ser | Ile | Pro | Thr | Thr | Thr | Ile | Arg | Arg | His | |
| | | | 130 | | | | 135 | | | | | 140 | | | | |
| GTC | GAT | TTG | CTC | GTT | GGG | GCG | GCT | GCT | TTC | TGT | TCC | GCT | ATG | TAC | GTG | 480 |
| Val | Asp | Leu | Leu | Val | Gly | Ala | Ala | Ala | Phe | Cys | Ser | Ala | Met | Tyr | Val | |
| | | | | | 150 | | | | | 155 | | | | | 160 | |
| GGG | GAT | CTC | TGC | GGA | TCT | GTC | TTC | CTC | GTC | TCC | CAG | CTG | TTC | ACC | ATC | 528 |
| Gly | Asp | Leu | Cys | Gly | Ser | Val | Phe | Leu | Val | Ser | Gln | Leu | Phe | Thr | Ile | |
| | | | | 165 | | | | | 170 | | | | | 175 | | |
| TCG | CCT | CGC | CGG | CAT | GAG | ACG | GTG | CAG | GAC | TGC | AAT | TGC | TCA | ATC | TAT | 576 |
| Ser | Pro | Arg | Arg | His | Glu | Thr | Val | Gln | Asp | Cys | Asn | Cys | Ser | Ile | Tyr | |
| | | | 180 | | | | | 185 | | | | | 190 | | | |
| CCC | GGC | CAC | ATA | ACG | GGT | CAC | CGT | ATG | GCT | TGG | GAT | ATG | ATG | ATG | AAC | 624 |
| Pro | Gly | His | Ile | Thr | Gly | His | Arg | Met | Ala | Trp | Asp | Met | Met | Met | Asn | |
| | | | 195 | | | | 200 | | | | | 205 | | | | |
| TGG | TAC | TAATAG | | | | | | | | | | | | | | 640 |
| Trp | Tyr | | | | | | | | | | | | | | | |
| | 210 | | | | | | | | | | | | | | | |

(2) INFORMATION FOR SEQ ID NO: 14:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 210 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Met | Leu | Gly | Lys | Ala | Ile | Asp | Thr | Leu | Thr | Cys | Gly | Phe | Ala | Asp | Leu | |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | | |
| Val | Gly | Tyr | Ile | Pro | Leu | Val | Gly | Ala | Pro | Leu | Gly | Gly | Ala | Ala | Arg | |
| | | | 20 | | | | | 25 | | | | | 30 | | | |
| Ala | Leu | Ala | His | Gly | Val | Arg | Val | Leu | Glu | Asp | Gly | Val | Asn | Tyr | Ala | |
| | | | 35 | | | | 40 | | | | | 45 | | | | |
| Thr | Gly | Asn | Leu | Pro | Gly | Cys | Ser | Phe | Ser | Ile | Phe | Leu | Leu | Ala | Leu | |
| | | | 50 | | | 55 | | | | | 60 | | | | | |
| Leu | Ser | Cys | Leu | Thr | Ile | Pro | Ala | Ser | Ala | Tyr | Glu | Val | Arg | Asn | Val | |
| | | | 65 | | 70 | | | | | 75 | | | | 80 | | |
| Ser | Gly | Met | Tyr | His | Val | Thr | Asn | Asp | Cys | Ser | Asn | Ser | Ser | Ile | Val | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | 85 | | 90 | | 95 | | | | | | | | | | |
| Tyr | Glu | Ala | Ala | Asp | Met | Ile | Met | His | Thr | Pro | Gly | Cys | Val | Pro | Cys |
| | | 100 | | | | | | 105 | | | | | 110 | | |
| Val | Arg | Glu | Asn | Asn | Ser | Ser | Arg | Cys | Trp | Val | Ala | Leu | Thr | Pro | Thr |
| | | 115 | | | | | 120 | | | | | 125 | | | |
| Leu | Ala | Ala | Arg | Asn | Ala | Ser | Ile | Pro | Thr | Thr | Thr | Ile | Arg | Arg | His |
| | | 130 | | | | 135 | | | | | 140 | | | | |
| Val | Asp | Leu | Leu | Val | Gly | Ala | Ala | Ala | Phe | Cys | Ser | Ala | Met | Tyr | Val |
| 145 | | | | | 150 | | | | | 155 | | | | | 160 |
| Gly | Asp | Leu | Cys | Gly | Ser | Val | Phe | Leu | Val | Ser | Gln | Leu | Phe | Thr | Ile |
| | | | | 165 | | | | | 170 | | | | | 175 | |
| Ser | Pro | Arg | Arg | His | Glu | Thr | Val | Gln | Asp | Cys | Asn | Cys | Ser | Ile | Tyr |
| | | | 180 | | | | | 185 | | | | | 190 | | |
| Pro | Gly | His | Ile | Thr | Gly | His | Arg | Met | Ala | Trp | Asp | Met | Met | Met | Asn |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| Trp | Tyr | | | | | | | | | | | | | | |
| | 210 | | | | | | | | | | | | | | |

(2) INFORMATION FOR SEQ ID NO: 15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

ATGCCCGGTT GCTCTTTCTC TATCTT

26

(2) INFORMATION FOR SEQ ID NO: 16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

ATGTTGGGTA AGGTCATCGA TACCCT

26

(2) INFORMATION FOR SEQ ID NO: 17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CTATTAGGAC CAGTTCATCA TCATATCCCA

30

(2) INFORMATION FOR SEQ ID NO: 18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CTATTACCAG TTCATCATCA TATCCCA

27

(2) INFORMATION FOR SEQ ID NO: 19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 36 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

ATACGACGCC ACGTCGATTC CCAGCTGTTC ACCATC

36

(2) INFORMATION FOR SEQ ID NO: 20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 36 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GATGGTGAAC AGCTGGGAAT CGACGTGGCG TCGTAT

36

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 723 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..720

(ix) FEATURE:
 (A) NAME/KEY: mat_peptide
 (B) LOCATION: 1..717

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

| | |
|---|-----|
| ATG TTG GGT AAG GTC ATC GAT ACC CTT ACA TGC GGC TTC GCC GAC CTC | 48 |
| Met Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu | |
| 1 5 10 15 | |
| GTG GGG TAC ATT CCG CTC GTC GGC GCC CCC CTA GGG GGC GCT GCC AGG | 96 |
| Val Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg | |
| 20 25 30 | |
| GCC CTG GCG CAT GGC GTC CGG GTT CTG GAG GAC GGC GTG AAC TAT GCA | 144 |
| Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala | |
| 35 40 45 | |
| ACA GGG AAT TTG CCC GGT TGC TCT TTC TCT ATC TTC CTC TTG GCT TTG | 192 |
| Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu | |
| 50 55 60 | |
| CTG TCC TGT CTG ACC GTT CCA GCT TCC GCT TAT GAA GTG CGC AAC GTG | 240 |
| Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr Glu Val Arg Asn Val | |
| 65 70 75 80 | |
| TCC GGG ATG TAC CAT GTC ACG AAC GAC TGC TCC AAC TCA AGC ATT GTG | 288 |
| Ser Gly Met Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val | |
| 85 90 95 | |
| TAT GAG GCA GCG GAC ATG ATC ATG CAC ACC CCC GGG TGC GTG CCC TGC | 336 |
| Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys | |
| 100 105 110 | |
| GTT CGG GAG AAC AAC TCT TCC CGC TGC TGG GTA GCG CTC ACC CCC ACG | 384 |
| Val Arg Glu Asn Asn Ser Ser Arg Cys Trp Val Ala Leu Thr Pro Thr | |
| 115 120 125 | |
| CTC GCA GCT AGG AAC GCC AGC GTC CCC ACC ACG ACA ATA CGA CGC CAC | 432 |
| Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Thr Ile Arg Arg His | |
| 130 135 140 | |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------|-----|
| GTC | GAT | TCC | CAG | CTG | TTC | ACC | ATC | TCG | CCT | CGC | CGG | CAT | GAG | ACG | GTG | 480 |
| Val | Asp | Ser | Gln | Leu | Phe | Thr | Ile | Ser | Pro | Arg | Arg | His | Glu | Thr | Val | |
| 145 | | | | | 150 | | | | | 155 | | | | | 160 | |
| CAG | GAC | TGC | AAT | TGC | TCA | ATC | TAT | CCC | GGC | CAC | ATA | ACG | GGT | CAC | CGT | 528 |
| Gln | Asp | Cys | Asn | Cys | Ser | Ile | Tyr | Pro | Gly | His | Ile | Thr | Gly | His | Arg | |
| | | | 165 | | | | | | 170 | | | | | 175 | | |
| ATG | GCT | TGG | GAT | ATG | ATG | ATG | AAC | TGG | TCG | CCT | ACA | ACG | GCC | CTG | GTG | 576 |
| Met | Ala | Trp | Asp | Met | Met | Met | Asn | Trp | Ser | Pro | Thr | Thr | Ala | Leu | Val | |
| | | | 180 | | | | | 185 | | | | | 190 | | | |
| GTA | TCG | CAG | CTG | CTC | CGG | ATC | CCA | CAA | GCT | GTC | GTG | GAC | ATG | GTG | GCG | 624 |
| Val | Ser | Gln | Leu | Leu | Arg | Ile | Pro | Gln | Ala | Val | Val | Asp | Met | Val | Ala | |
| | | 195 | | | | | 200 | | | | | 205 | | | | |
| GGG | GCC | CAT | TGG | GGA | GTC | CTG | GCG | GGT | CTC | GCC | TAC | TAT | TCC | ATG | GTG | 672 |
| Gly | Ala | His | Trp | Gly | Val | Leu | Ala | Gly | Leu | Ala | Tyr | Tyr | Ser | Met | Val | |
| | 210 | | | | | 215 | | | | | 220 | | | | | |
| GGG | AAC | TGG | GCT | AAG | GTT | TTC | ATT | GTG | ATG | CTA | CTC | TTT | GCT | CCC | TAATAG | 723 |
| Gly | Asn | Trp | Ala | Lys | Val | Leu | Ile | Val | Met | Leu | Leu | Phe | Ala | Pro | | |
| 225 | | | | 230 | | | | | | 235 | | | | 240 | | |

(2) INFORMATION FOR SEQ ID NO: 22:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 239 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Leu | Gly | Lys | Val | Ile | Asp | Thr | Leu | Thr | Cys | Gly | Phe | Ala | Asp | Leu |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| Val | Gly | Tyr | Ile | Pro | Leu | Val | Gly | Ala | Pro | Leu | Gly | Gly | Ala | Ala | Arg |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| Ala | Leu | Ala | His | Gly | Val | Arg | Val | Leu | Glu | Asp | Gly | Val | Asn | Tyr | Ala |
| | | 35 | | | | 40 | | | | | | 45 | | | |
| Thr | Gly | Asn | Leu | Pro | Gly | Cys | Ser | Phe | Ser | Ile | Phe | Leu | Leu | Ala | Leu |
| | 50 | | | | 55 | | | | | | 60 | | | | |
| Leu | Ser | Cys | Leu | Thr | Val | Pro | Ala | Ser | Ala | Tyr | Glu | Val | Arg | Asn | Val |
| | 65 | | | 70 | | | | | | 75 | | | | 80 | |
| Ser | Gly | Met | Tyr | His | Val | Thr | Asn | Asp | Cys | Ser | Asn | Ser | Ser | Ile | Val |
| | | | 85 | | | | | 90 | | | | | | 95 | |
| Tyr | Glu | Ala | Ala | Asp | Met | Ile | Met | His | Thr | Pro | Gly | Cys | Val | Pro | Cys |
| | | 100 | | | | | 105 | | | | | | 110 | | |
| Val | Arg | Glu | Asn | Asn | Ser | Ser | Arg | Cys | Trp | Val | Ala | Leu | Thr | Pro | Thr |
| | | 115 | | | | | 120 | | | | | 125 | | | |
| Leu | Ala | Ala | Arg | Asn | Ala | Ser | Val | Pro | Thr | Thr | Thr | Ile | Arg | Arg | His |
| | 130 | | | | 135 | | | | | | 140 | | | | |
| Val | Asp | Ser | Gln | Leu | Phe | Thr | Ile | Ser | Pro | Arg | Arg | His | Glu | Thr | Val |
| | 145 | | | 150 | | | | | | 155 | | | | 160 | |
| Gln | Asp | Cys | Asn | Cys | Ser | Ile | Tyr | Pro | Gly | His | Ile | Thr | Gly | His | Arg |
| | | | 165 | | | | | | 170 | | | | | 175 | |

Met Ala Trp Asp Met Met Met Asn Trp Ser Pro Thr Thr Ala Leu Val
180 185 190
Val Ser Gln Leu Leu Arg Ile Pro Gln Ala Val Val Asp Met Val Ala
195 200 205
Gly Ala His Trp Gly Val Leu Ala Gly Leu Ala Tyr Tyr Ser Met Val
210 215 220
Gly Asn Trp Ala Lys Val Leu Ile Val Met Leu Leu Phe Ala Pro
225 230 235

(2) INFORMATION FOR SEQ ID NO: 23:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 561 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

- (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..558

- (ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..555

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

| | |
|---|-----|
| ATG TTG GGT AAG GTC ATC GAT ACC CTT ACA TGC GGC TTC GCC GAC CTC | 48 |
| Met Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu | |
| 1 5 10 15 | |
| GTG GGG TAC ATT CCG CTC GTC GGC GCC CCC CTA GGG GGC GCT GCC AGG | 96 |
| Val Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg | |
| 20 25 30 | |
| GCC CTG GCG CAT GGC GTC CGG GTT CTG GAG GAC GGC GTG AAC TAT GCA | 144 |
| Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala | |
| 35 40 45 | |
| ACA GGG AAT TTG CCC GGT TGC TCT TTC TCT ATC TTC CTC TTG GCT TTG | 192 |
| Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu | |
| 50 55 60 | |
| CTG TCC TGT CTG ACC GTT CCA GCT TCC GCT TAT GAA GTG CGC AAC GTG | 240 |
| Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr Glu Val Arg Asn Val | |
| 65 70 75 80 | |
| TCC GGG ATG TAC CAT GTC ACG AAC GAC TGC TCC AAC TCA AGC ATT GTG | 288 |
| Ser Gly Met Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val | |
| 85 90 95 | |
| TAT GAG GCA GCG GAC ATG ATC ATG CAC ACC CCC GGG TGC GTG CCC TGC | 336 |
| Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys | |
| 100 105 110 | |
| GTT CGG GAG AAC AAC TCT TCC CGC TGC TGG GTA GCG CTC ACC CCC ACC | 384 |
| Val Arg Glu Asn Asn Ser Ser Arg Cys Trp Val Ala Leu Thr Pro Thr | |

| | | | |
|---|-----|-----|-----|
| 115 | 120 | 125 | |
| CTC GCA GCT AGG AAC GCC AGC GTC CCC ACC ACG ACA ATA CGA CGC CAC | | | 432 |
| Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Thr Ile Arg Arg His | | | |
| 130 | 135 | 140 | |
| GTC GAT TCC CAG CTG TTC ACC ATC TCG CCT CGC CGG CAT GAG ACG GTG | | | 480 |
| Val Asp Ser Gln Leu Phe Thr Ile Ser Pro Arg Arg His Glu Thr Val | | | |
| 145 | 150 | 155 | 160 |
| CAG GAC TGC AAT TGC TCA ATC TAT CCC GGC CAC ATA ACG GGT CAC CGT | | | 528 |
| Gln Asp Cys Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg | | | |
| 165 | 170 | 175 | |
| ATG GCT TGG GAT ATG ATG ATG AAC TGG TAATAG | | | 561 |
| Met Ala Trp Asp Met Met Met Asn Trp | | | |
| 180 | 185 | | |

(2) INFORMATION FOR SEQ ID NO: 24:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 185 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Met Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu
 1 5 10 15
 Val Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg
 20 25 30
 Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala
 35 40 45
 Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu
 50 55 60
 Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr Glu Val Arg Asn Val
 65 70 75 80
 Ser Gly Met Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val
 85 90 95
 Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys
 100 105 110
 Val Arg Glu Asn Asn Ser Ser Arg Cys Trp Val Ala Leu Thr Pro Thr
 115 120 125
 Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Thr Ile Arg Arg His
 130 135 140
 Val Asp Ser Gln Leu Phe Thr Ile Ser Pro Arg Arg His Glu Thr Val
 145 150 155 160
 Gln Asp Cys Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg
 165 170 175
 Met Ala Trp Asp Met Met Met Asn Trp
 180 185

(2) INFORMATION FOR SEQ ID NO: 25:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 606 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 1..603

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
 (B) LOCATION: 1..600

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

| | |
|---|-----|
| ATG TTG GGT AAG GTC ATC GAT ACC CTT ACA TGC GGC TTC GCC GAC CTC | 48 |
| Met Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu | |
| 1 5 10 15 | |
| GTG GGG TAC ATT CCG CTC GTC GGC GCC CCC CTA GGG GGC GCT GCC AGG | 96 |
| Val Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg | |
| 20 25 30 | |
| GCC CTG GCG CAT GGC GTC CGG GTT CTG GAG GAC GGC GTG AAC TAT GCA | 144 |
| Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala | |
| 35 40 45 | |
| ACA GGG AAT TTG CCC GGT TGC TCT TTC TCT ATC TTC CTC TTG GCT TTG | 192 |
| Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu | |
| 50 55 60 | |
| CTG TCC TGT CTG ACC GTT CCA GCT TCC GCT TAT GAA GTG CGC AAC GTG | 240 |
| Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr Glu Val Arg Asn Val | |
| 65 70 75 80 | |
| TCC GGG ATG TAC CAT GTC ACG AAC GAC TGC TCC AAC TCA AGC ATT GTG | 288 |
| Ser Gly Met Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val | |
| 85 90 95 | |
| TAT GAG GCA GCG GAC ATG ATC ATG CAC ACC CCC GGG TGC GTG CCC TGC | 336 |
| Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys | |
| 100 105 110 | |
| GTT CGG GAG AAC AAC TCT TCC CGC TGC TGG GTA GCG CTC ACC CCC ACG | 384 |
| Val Arg Glu Asn Asn Ser Ser Arg Cys Trp Val Ala Leu Thr Pro Thr | |
| 115 120 125 | |
| CTC GCA GCT AGG AAC GCC AGC GTC CCC ACC ACG ACA ATA CGA CGC CAC | 432 |
| Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Thr Ile Arg Arg His | |
| 130 135 140 | |
| GTC GAT TCC CAG CTG TTC ACC ATC TCG CCT CGC CGG CAT GAG ACG GTG | 480 |
| Val Asp Ser Gln Leu Phe Thr Ile Ser Pro Arg Arg His Glu Thr Val | |
| 145 150 155 160 | |
| CAG GAC TGC AAT TGC TCA ATC TAT CCC GGC CAC ATA ACG GGT CAC CGT | 528 |
| Gln Asp Cys Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg | |
| 165 170 175 | |
| ATG GCT TGG GAT ATG ATG ATG AAC TGG TGG CCT ACA ACG GCC CTG GTG | 576 |

Met Ala Trp Asp Met Met Met Asn Trp Ser Pro Thr Thr Ala Leu Val
180 185 190

GTA TCG CAG CTG CTC CGG ATC CTC TAATAG
Val Ser Gln Leu Leu Arg Ile Leu
195 200

606

(2) INFORMATION FOR SEQ ID NO: 26:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 200 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Met Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu
1 5 10 15
Val Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg
20 25 30
Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala
35 40 45
Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu
50 55 60
Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr Glu Val Arg Asn Val
65 70 75 80
Ser Gly Met Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val
85 90 95
Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys
100 105 110
Val Arg Glu Asn Asn Ser Ser Arg Cys Trp Val Ala Leu Thr Pro Thr
115 120 125
Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Thr Ile Arg Arg His
130 135 140
Val Asp Ser Gln Leu Phe Thr Ile Ser Pro Arg Arg His Glu Thr Val
145 150 155 160
Gln Asp Cys Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg
165 170 175
Met Ala Trp Asp Met Met Met Asn Trp Ser Pro Thr Thr Ala Leu Val
180 185 190
Val Ser Gln Leu Leu Arg Ile Leu
195 200

(2) INFORMATION FOR SEQ ID NO: 27:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 636 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..633

(ix) FEATURE:

(A) NAME/KEY: mat_peptide

(B) LOCATION: 1..630

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

| | |
|---|-----|
| ATG TTG GGT AAG GTC ATC GAT ACC CTT ACA TGC GGC TTC GCC GAC CTC | 48 |
| Met Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu | |
| 1 5 10 15 | |
| GTG GGG TAC ATT CCG CTC GTC GGC GCC CCC CTA GGG GGC GCT GCC AGG | 96 |
| Val Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg | |
| 20 25 30 | |
| GCC CTG GCG CAT GGC GTC CGG GTT CTG GAG GAC GGC GTG AAC TAT GCA | 144 |
| Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala | |
| 35 40 45 | |
| ACA GGG AAT TTG CCC GGT TGC TCT TTC TCT ATC TTC CTC TTG GCT TTG | 192 |
| Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu | |
| 50 55 60 | |
| CTG TCC TGT CTG ACC GTT CCA GCT TCC GCT TAT GAA GTG CGC AAC GTG | 240 |
| Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr Glu Val Arg Asn Val | |
| 65 70 75 80 | |
| TCC GGG ATG TAC CAT GTC ACG AAC GAC TGC TCC AAC TCA AGC ATT GTG | 288 |
| Ser Gly Met Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val | |
| 85 90 95 | |
| TAT GAG GCA GCG GAC ATG ATC ATG CAC ACC CCC GGG TGC GTG CCC TGC | 336 |
| Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys | |
| 100 105 110 | |
| GTT CGG GAG AAC AAC TCT TCC CGC TGC TGG GTA GCG CTC ACC CCC ACG | 384 |
| Val Arg Glu Asn Asn Ser Ser Arg Cys Trp Val Ala Leu Thr Pro Thr | |
| 115 120 125 | |
| CTC GCA GCT AGG AAC GCC AGC GTC CCC ACC ACG ACA ATA CGA CGC CAC | 432 |
| Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Thr Ile Arg Arg His | |
| 130 135 140 | |
| GTC GAT TCC CAG CTG TTC ACC ATC TCG CCT CGC CGG CAT GAG ACG GTG | 480 |
| Val Asp Ser Gln Leu Phe Thr Ile Ser Pro Arg Arg His Glu Thr Val | |
| 145 150 155 160 | |
| CAG GAC TGC AAT TGC TCA ATC TAT CCC GGC CAC ATA ACG GGT CAC CGT | 528 |
| Gln Asp Cys Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg | |
| 165 170 175 | |
| ATG GCT TGG GAT ATG ATG ATG AAC TGG TCG CCT ACA ACG GCC CTG GTG | 576 |
| Met Ala Trp Asp Met Met Met Asn Trp Ser Pro Thr Thr Ala Leu Val | |
| 180 185 190 | |
| GTA TCG CAG CTG CTC CGG ATC GTG ATC GAG GGC AGA CAC CAT CAC CAC | 624 |
| Val Ser Gln Leu Leu Arg Ile Val Ile Glu Gly Arg His His His His | |
| 195 200 205 | |
| CAT CAC TAATAG | 636 |

His His
210

(2) INFORMATION FOR SEQ ID NO: 28:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 210 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Met Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu
1 5 10 15
Val Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg
20 25 30
Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala
35 40 45
Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu
50 55 60
Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr Glu Val Arg Asn Val
65 70 75 80
Ser Gly Met Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val
85 90 95
Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys
100 105 110
Val Arg Glu Asn Asn Ser Ser Arg Cys Trp Val Ala Leu Thr Pro Thr
115 120 125
Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Thr Ile Arg Arg His
130 135 140
Val Asp Ser Gln Leu Phe Thr Ile Ser Pro Arg Arg His Glu Thr Val
145 150 155 160
Gln Asp Cys Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg
165 170 175
Met Ala Trp Asp Met Met Met Asn Trp Ser Pro Thr Thr Ala Leu Val
180 185 190
Val Ser Gln Leu Leu Arg Ile Val Ile Glu Gly Arg His His His His
195 200 205
His His
210

(2) INFORMATION FOR SEQ ID NO: 29:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 630 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..627

(ix) FEATURE:

(A) NAME/KEY: mat_peptide

(B) LOCATION: 1..624

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

| | |
|---|-----|
| ATG GGT AAG GTC ATC GAT ACC CTT ACG TGC GGA TTC GCC GAT CTC ATG | 48 |
| Met Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu Met | |
| 1 5 10 15 | |
| GGG TAC ATC CCG CTC GTC GGC GCT CCC GTA GGA GGC GTC GCA AGA GCC | 96 |
| Gly Tyr Ile Pro Leu Val Gly Ala Pro Val Gly Gly Val Ala Arg Ala | |
| 20 25 30 | |
| CTT GCG CAT GGC GTG AGG GCC CTT GAA GAC GGG ATA AAT TTC GCA ACA | 144 |
| Leu Ala His Gly Val Arg Ala Leu Glu Asp Gly Ile Asn Phe Ala Thr | |
| 35 40 45 | |
| GGG AAT TTG CCC GGT TGC TCC TTT TCT ATT TTC CTT CTC GCT CTG TTC | 192 |
| Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu Phe | |
| 50 55 60 | |
| TCT TGC TTA ATT CAT CCA GCA GCT AGT CTA GAG TGG CGG AAT ACG TCT | 240 |
| Ser Cys Leu Ile His Pro Ala Ala Ser Leu Glu Trp Arg Asn Thr Ser | |
| 65 70 75 80 | |
| GGC CTC TAT GTC CTT ACC AAC GAC TGT TCC AAT AGC AGT ATT GTG TAC | 288 |
| Gly Leu Tyr Val Leu Thr Asn Asp Cys Ser Asn Ser Ser Ile Val Tyr | |
| 85 90 95 | |
| GAG GCC GAT GAC GTT ATT CTG CAC ACA CCC GGC TGC ATA CCT TGT GTC | 336 |
| Glu Ala Asp Asp Val Ile Leu His Thr Pro Gly Cys Ile Pro Cys Val | |
| 100 105 110 | |
| CAG GAC GGC AAT ACA TCC ACG TGC TGG ACC CCA GTG ACA CCT ACA GTG | 384 |
| Gln Asp Gly Asn Thr Ser Thr Cys Trp Thr Pro Val Thr Pro Thr Val | |
| 115 120 125 | |
| GCA GTC AAG TAC GTC GGA GCA ACC ACC GCT TCG ATA CGC AGT CAT GTG | 432 |
| Ala Val Lys Tyr Val Gly Ala Thr Thr Ala Ser Ile Arg Ser His Val | |
| 130 135 140 | |
| GAC CTA TTA GTG GGC GCG GCC ACG ATG TSC TCT GCG CTC TAC GTG GGT | 480 |
| Asp Leu Leu Val Gly Ala Ala Thr Met Cys Ser Ala Leu Tyr Val Gly | |
| 145 150 155 160 | |
| GAC ATG TGT GGG GCT GTC TTC CTC GTG GGA CAA GCC TTC ACG TTC AGA | 528 |
| Asp Met Cys Gly Ala Val Phe Leu Val Gly Gln Ala Phe Thr Phe Arg | |
| 165 170 175 | |
| CCT CGT CGC CAT CAA ACG GTC CAG ACC TGT AAC TGC TCG CTG TAC CCA | 576 |
| Pro Arg Arg His Gln Thr Val Gln Thr Cys Asn Cys Ser Leu Tyr Pro | |
| 180 185 190 | |
| GGC CAT CTT TCA GGA CAT CGA ATG GCT TGG GAT ATG ATG ATG AAC TGG | 624 |
| Gly His Leu Ser Gly His Arg Met Ala Trp Asp Met Met Met Asn Trp | |
| 195 200 205 | |
| TAATAG | 636 |

(2) INFORMATION FOR SEQ ID NO: 30:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 208 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

```

Met Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu Met
 1           5           10           15
Gly Tyr Ile Pro Leu Val Gly Ala Pro Val Gly Gly Val Ala Arg Ala
          20           25           30
Leu Ala His Gly Val Arg Ala Leu Glu Asp Gly Ile Asn Phe Ala Thr
          35           40           45
Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu Phe
          50           55           60
Ser Cys Leu Ile His Pro Ala Ala Ser Leu Glu Trp Arg Asn Thr Ser
 65           70           75           80
Gly Leu Tyr Val Leu Thr Asn Asp Cys Ser Asn Ser Ser Ile Val Tyr
          85           90           95
Glu Ala Asp Asp Val Ile Leu His Thr Pro Gly Cys Ile Pro Cys Val
          100          105          110
Gln Asp Gly Asn Thr Ser Thr Cys Trp Thr Pro Val Thr Pro Thr Val
          115          120          125
Ala Val Lys Tyr Val Gly Ala Thr Thr Ala Ser Ile Arg Ser His Val
          130          135          140
Asp Leu Leu Val Gly Ala Ala Thr Met Cys Ser Ala Leu Tyr Val Gly
145          150          155          160
Asp Met Cys Gly Ala Val Phe Leu Val Gly Gln Ala Phe Thr Phe Arg
          165          170          175
Pro Arg Arg His Gln Thr Val Gln Thr Cys Asn Cys Ser Leu Tyr Pro
          180          185          190
Gly His Leu Ser Gly His Arg Met Ala Trp Asp Met Met Met Asn Trp
          195          200          205
  
```

(2) INFORMATION FOR SEQ ID NO: 31:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 630 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..627

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..624

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

| | |
|---|-----|
| ATG GGT AAG GTC ATC GAT ACC CTA ACG TGC GGA TTC GCC GAT CTC ATG | 48 |
| Met Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu Met | |
| 1 5 10 15 | |
| GGG TAT ATC CCG CTC GTA GGC GGC CCC ATT GGG GGC GTC GCA AGG GCT | 96 |
| Gly Tyr Ile Pro Leu Val Gly Gly Pro Ile Gly Gly Val Ala Arg Ala | |
| 20 25 30 | |
| CTC GCA CAC GGT GTG AGG GTC CTT GAG GAC GGG GTA AAC TAT GCA ACA | 144 |
| Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala Thr | |
| 35 40 45 | |
| GGG AAT TTA CCC GGT TGC TCT TTC TCT ATC TTT ATT CTT GCT CTT CTC | 192 |
| Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Ile Leu Ala Leu Leu | |
| 50 55 60 | |
| TCG TGT CTG ACC GTT CCG GCC TCT GCA GTT CCC TAC CGA AAT GCC TCT | 240 |
| Ser Cys Leu Thr Val Pro Ala Ser Ala Val Pro Tyr Arg Asn Ala Ser | |
| 65 70 75 80 | |
| GGG ATT TAT CAT GTT ACC AAT GAT TGC CCA AAC TCT TCC ATA GTC TAT | 288 |
| Gly Ile Tyr His Val Thr Asn Asp Cys Pro Asn Ser Ser Ile Val Tyr | |
| 85 90 95 | |
| GAG GCA GAT AAC CTG ATC CTA CAC GCA CCT GGT TGC GTG CCT TGT GTC | 336 |
| Glu Ala Asp Asn Leu Ile Leu His Ala Pro Gly Cys Val Pro Cys Val | |
| 100 105 110 | |
| ATG ACA GGT AAT GTG AGT AGA TGC TGG GTC CAA ATT ACC CCT ACA CTG | 384 |
| Met Thr Gly Asn Val Ser Arg Cys Trp Val Gln Ile Thr Pro Thr Leu | |
| 115 120 125 | |
| TCA GCC CCG AGC CTC GGA GCA GTC ACG GCT CCT CTT CGG AGA GCC GTT | 432 |
| Ser Ala Pro Ser Leu Gly Ala Val Thr Ala Pro Leu Arg Arg Ala Val | |
| 130 135 140 | |
| GAC TAC CTA GCG GGA GGG GCT GCC CTC TGC TCC GCG TTA TAC GTA GGA | 480 |
| Asp Tyr Leu Ala Gly Gly Ala Ala Leu Cys Ser Ala Leu Tyr Val Gly | |
| 145 150 155 160 | |
| GAC GCG TGT GGG GCA CTA TTC TTG GTA GGC CAA ATG TTC ACC TAT AGG | 528 |
| Asp Ala Cys Gly Ala Leu Phe Leu Val Gly Gln Met Phe Thr Tyr Arg | |
| 165 170 175 | |
| CCT CGC CAG CAC GCT ACG GTG CAG AAC TGC AAC TGT TCC ATT TAC AGT | 576 |
| Pro Arg Gln His Ala Thr Val Gln Asn Cys Asn Cys Ser Ile Tyr Ser | |
| 180 185 190 | |
| GGC CAT GTT ACC GGC CAC CGG ATG GCA TGG GAT ATG ATG ATG AAC TGG | 624 |
| Gly His Val Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn Trp | |
| 195 200 205 | |
| TAATAG | 630 |

(2) INFORMATION FOR SEQ ID NO: 32:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 208 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Met Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu Met
 1 5 10 15
 Gly Tyr Ile Pro Leu Val Gly Gly Pro Ile Gly Gly Val Ala Arg Ala
 20 25 30
 Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala Thr
 35 40 45
 Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Ile Leu Ala Leu Leu
 50 55 60
 Ser Cys Leu Thr Val Pro Ala Ser Ala Val Pro Tyr Arg Asn Ala Ser
 65 70 75 80
 Gly Ile Tyr His Val Thr Asn Asp Cys Pro Asn Ser Ser Ile Val Tyr
 85 90 95
 Glu Ala Asp Asn Leu Ile Leu His Ala Pro Gly Cys Val Pro Cys Val
 100 105 110
 Met Thr Gly Asn Val Ser Arg Cys Trp Val Gln Ile Thr Pro Thr Leu
 115 120 125
 Ser Ala Pro Ser Leu Gly Ala Val Thr Ala Pro Leu Arg Arg Ala Val
 130 135 140
 Asp Tyr Leu Ala Gly Gly Ala Ala Leu Cys Ser Ala Leu Tyr Val Gly
 145 150 155 160
 Asp Ala Cys Gly Ala Leu Phe Leu Val Gly Gln Met Phe Thr Tyr Arg
 165 170 175
 Pro Arg Gln His Ala Thr Val Gln Asn Cys Asn Cys Ser Ile Tyr Ser
 180 185 190
 Gly His Val Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn Trp
 195 200 205

(2) INFORMATION FOR SEQ ID NO: 33:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

TGGGATATGA TGATGAACTG GTC

23

(2) INFORMATION FOR SEQ ID NO: 34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CTATTATGGT GGTAAGCCAC AGAGCAGGAG

30

(2) INFORMATION FOR SEQ ID NO: 35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1476 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1473

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 1..1470

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

| | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|-----|
| TGG | GAT | ATG | ATG | ATG | AAC | TGG | TCG | CCT | ACA | ACG | GCC | CTG | GTG | GTA | TCG | | 48 |
| Trp | Asp | Met | Met | Met | Asn | Trp | Ser | Pro | Thr | Thr | Ala | Leu | Val | Val | Ser | | |
| 1 | | | | | 5 | | | | 10 | | | | | 15 | | | |
| CAG | CTG | CTC | CGG | ATC | CCA | CAA | GCT | GTC | GTG | GAC | ATG | GTG | GCG | GGG | GCC | | 96 |
| Gln | Leu | Leu | Arg | Ile | Pro | Gln | Ala | Val | Val | Asp | Met | Val | Ala | Gly | Ala | | |
| | | | 20 | | | | | 25 | | | | | 30 | | | | |
| CAT | TGG | GGA | GTC | CTG | GCG | GGC | CTC | GCC | TAC | TAT | TCC | ATG | GTG | GGG | AAC | | 144 |
| His | Trp | Gly | Val | Leu | Ala | Gly | Leu | Ala | Tyr | Tyr | Ser | Met | Val | Gly | Asn | | |
| | | | 35 | | | | 40 | | | | | 45 | | | | | |
| TGG | GCT | AAG | GTT | TTG | GTT | GTG | ATG | CTA | CTC | TTT | GCC | GGC | GTC | GAC | GGG | | 192 |
| Trp | Ala | Lys | Val | Leu | Val | Val | Met | Leu | Leu | Phe | Ala | Gly | Val | Asp | Gly | | |
| | | | 50 | | | | 55 | | | | | 60 | | | | | |
| CAT | ACC | CGC | GTG | TCA | GGA | GGG | GCA | GCA | GCC | TCC | GAT | ACC | AGG | GGC | CTT | | 240 |
| His | Thr | Arg | Val | Ser | Gly | Gly | Ala | Ala | Ala | Ser | Asp | Thr | Arg | Gly | Leu | | |
| | | | 65 | | | 70 | | | | 75 | | | | | 80 | | |
| GTG | TCC | CTC | TTT | AGC | CCC | GGG | TCG | GCT | CAG | AAA | ATC | CAG | CTC | GTA | AAC | | 288 |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| Val | Ser | Leu | Phe | Ser | Pro | Gly | Ser | Ala | Gln | Lys | Ile | Gln | Leu | Val | Asn | |
| | | | | 85 | | | | | 90 | | | | | 95 | | |
| ACC | AAC | GGC | AGT | TGG | CAC | ATC | AAC | AGG | ACT | GCC | CTG | AAC | TGC | AAC | GAC | 336 |
| Thr | Asn | Gly | Ser | Trp | His | Ile | Asn | Arg | Thr | Ala | Leu | Asn | Cys | Asn | Asp | |
| | | | 100 | | | | | 105 | | | | | 110 | | | |
| TCC | CTC | CAA | ACA | GGG | TTC | TTT | GCC | GCA | CTA | TTC | TAC | AAA | CAC | AAA | TTC | 384 |
| Ser | Leu | | Thr | Gly | Phe | Phe | Ala | Ala | Leu | Phe | Tyr | Lys | His | Lys | Phe | |
| | | 115 | | | | | 120 | | | | | 125 | | | | |
| AAC | TCG | TCT | GGA | TGC | CCA | GAG | CGC | TTG | GCC | AGC | TGT | CGC | TCC | ATC | GAC | 432 |
| Asn | Ser | Ser | Gly | Cys | Pro | Glu | Arg | Leu | Ala | Ser | Cys | Arg | Ser | Ile | Asp | |
| | | 130 | | | | 135 | | | | | 140 | | | | | |
| AAG | TTC | GCT | CAG | GGG | TGG | GGT | CCC | CTC | ACT | TAC | ACT | GAG | CCT | AAC | AGC | 480 |
| Lys | Phe | Ala | Gln | Gly | Trp | Gly | Pro | Leu | Thr | Tyr | Thr | Glu | Pro | Asn | Ser | |
| | | | | | 150 | | | | | 155 | | | | | 160 | |
| TCG | GAC | CAG | AGG | CCC | TAC | TGC | TGG | CAC | TAC | GCG | CCT | CGA | CCG | TGT | GGT | 528 |
| Ser | Asp | Gln | Arg | Pro | Tyr | Cys | Trp | His | Tyr | Ala | Pro | Arg | Pro | Cys | Gly | |
| | | | | 165 | | | | | 170 | | | | | 175 | | |
| ATT | GTA | CCC | GCG | TCT | CAG | GTG | TGC | GGT | CCA | GTG | TAT | TGC | TTC | ACC | CCG | 576 |
| Ile | Val | Pro | Ala | Ser | Gln | Val | Cys | Gly | Pro | Val | Tyr | Cys | Phe | Thr | Pro | |
| | | | 180 | | | | | 185 | | | | | 190 | | | |
| AGC | CCT | GTT | GTG | GTG | GGG | ACG | ACC | GAT | CGG | TTT | GGT | GTC | CCC | ACG | TAT | 624 |
| Ser | Pro | Val | Val | Val | Gly | Thr | Asp | Arg | Phe | Gly | Val | Pro | Thr | Tyr | | |
| | | 195 | | | | 200 | | | | | 205 | | | | | |
| AAC | TGG | GGG | GCG | AAC | GAC | TCG | GAT | GTG | CTG | ATT | CTC | AAC | AAC | ACG | CGG | 672 |
| Asn | Trp | Gly | Ala | Asn | Asp | Ser | Asp | Val | Leu | Ile | Leu | Asn | Asn | Thr | Arg | |
| | | 210 | | | | 215 | | | | | 220 | | | | | |
| CCG | CCG | CGA | GGC | AAC | TGG | TTC | GGC | TGT | ACA | TGG | ATG | AAT | GGC | ACT | GGG | 720 |
| Pro | Pro | Arg | Gly | Asn | Trp | Phe | Gly | Cys | Thr | Trp | Met | Asn | Gly | Thr | Gly | |
| | | 225 | | | 230 | | | | 235 | | | | | 240 | | |
| TTC | ACC | AAG | ACG | TGT | GGG | GGC | CCC | CCG | TGC | AAC | ATC | GGG | GGG | GCC | GGC | 768 |
| Phe | Thr | Lys | Thr | Cys | Gly | Gly | Pro | Pro | Cys | Asn | Ile | Gly | Gly | Ala | Gly | |
| | | | 245 | | | | | | 250 | | | | | 255 | | |
| AAC | AAC | ACC | TTG | ACC | TGC | CCC | ACT | GAC | TGT | TTT | CGG | AAG | CAC | CCC | GAG | 816 |
| Asn | Asn | Thr | Leu | Thr | Cys | Pro | Thr | Asp | Cys | Phe | Arg | Lys | His | Pro | Glu | |
| | | | 260 | | | | | 265 | | | | | 270 | | | |
| GCC | ACC | TAC | GCC | AGA | TGC | GGT | TCT | GGG | CCC | TGG | CTG | ACA | CCT | AGG | TGT | 864 |
| Ala | Thr | Tyr | Ala | Arg | Cys | Gly | Ser | Gly | Pro | Trp | Leu | Thr | Pro | Arg | Cys | |
| | | 275 | | | | 280 | | | | | | 285 | | | | |
| ATG | GTT | CAT | TAC | CCA | TAT | AGG | CTC | TGG | CAC | TAC | CCC | TGC | ACT | GTC | AAC | 912 |
| Met | Val | His | Tyr | Pro | Tyr | Arg | Leu | Trp | His | Tyr | Pro | Cys | Thr | Val | Asn | |
| | | 290 | | | | 295 | | | | | 300 | | | | | |
| TTC | ACC | ATC | TTC | AAG | GTT | AGG | ATG | TAC | GTG | GGG | GGC | GTG | GAG | CAC | AGG | 960 |
| Phe | Thr | Ile | Phe | Lys | Val | Arg | Met | Tyr | Val | Gly | Gly | Val | Glu | His | Arg | |
| | | 305 | | | 310 | | | | 315 | | | | | 320 | | |
| TTC | GAA | GCC | GCA | TGC | AAT | TGG | ACT | CGA | GGA | GAG | CGT | TGT | GAC | TTG | GAG | 1008 |
| Phe | Glu | Ala | Ala | Cys | Asn | Trp | Thr | Arg | Gly | Glu | Arg | Cys | Asp | Leu | Glu | |
| | | | 325 | | | | | | 330 | | | | 335 | | | |
| GAC | AGG | GAT | AGA | TCA | GAG | CTT | AGC | CCG | CTG | CTG | CTG | TCT | ACA | ACA | GAG | 1056 |
| Asp | Arg | Asp | Arg | Ser | Glu | Leu | Ser | Pro | Leu | Leu | Leu | Ser | Thr | Thr | Glu | |
| | | | 340 | | | | | 345 | | | | | 350 | | | |
| TGG | CAG | ATA | CTG | CCC | TGT | TCC | TTC | ACC | ACC | CTG | CCG | GCC | CTA | TCC | ACC | 1104 |
| Trp | Gln | Ile | Leu | Pro | Cys | Ser | Phe | Thr | Thr | Leu | Pro | Ala | Leu | Ser | Thr | |

| 355 | 360 | 365 | |
|---|-----|-----|------|
| GGC CTG ATC CAC CTC CAT CAG AAC ATC GTG GAC GTG CAA TAC CTG TAC Gly Leu Ile His Leu His Gln Asn Ile Val Asp Val Gln Tyr Leu Tyr 370 375 380 | | | 1152 |
| GGT GTA GGG TCG GCG GTT GTC TCC CTT GTC ATC AAA TGG GAG TAT GTC Gly Val Gly Ser Ala Val Val Ser Leu Val Ile Lys Trp Glu Tyr Val 385 390 395 400 | | | 1200 |
| CTG TTG CTC TTC CTT CTC CTG GCA GAC GCG CGC ATC TGC GCC TGC TTA Leu Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Ile Cys Ala Cys Leu 405 410 415 | | | 1248 |
| TGG ATG ATG CTG CTG ATA GCT CAA GCT GAG GCC GCC TTA GAG AAC CTG Trp Met Met Leu Leu Ile Ala Gln Ala Glu Ala Ala Leu Glu Asn Leu 420 425 430 | | | 1296 |
| GTG GTC CTC AAT GCG GCG GCC GTG GCC GGG GCG CAT GGC ACT CTT TCC Val Val Leu Asn Ala Ala Ala Val Ala Gly Ala His Gly Thr Leu Ser 435 440 445 | | | 1344 |
| TTC CTT GTG TTC TTC TGT GCT GCC TGG TAC ATC AAG GGC AGG CTG GTC Phe Leu Val Phe Phe Cys Ala Ala Trp Tyr Ile Lys Gly Arg Leu Val 450 455 460 | | | 1392 |
| CCT GGT GCG GCA TAC GCC TTC TAT GGC GTG TGG CCG CTG CTC CTG CTT Pro Gly Ala Ala Tyr Ala Phe Tyr Gly Val Trp Pro Leu Leu Leu Leu 465 470 475 480 | | | 1440 |
| CTG CTG GCC TTA CCA CCA CGA GCT TAT GCC TAGTAA Leu Leu Ala Leu Pro Pro Arg Ala Tyr Ala 485 490 | | | 1476 |

(2) INFORMATION FOR SEQ ID NO: 36:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 490 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

Trp Asp Met Met Met Asn Trp Ser Pro Thr Thr Ala Leu Val Val Ser
1 5 10 15
Gln Leu Leu Arg Ile Pro Gln Ala Val Val Asp Met Val Ala Gly Ala
20 25 30
His Trp Gly Val Leu Ala Gly Leu Ala Tyr Tyr Ser Met Val Gly Asn
35 40 45
Trp Ala Lys Val Leu Val Val Met Leu Leu Phe Ala Gly Val Asp Gly
50 55 60
His Thr Arg Val Ser Gly Gly Ala Ala Ala Ser Asp Thr Arg Gly Leu
65 70 75 80
Val Ser Leu Phe Ser Pro Gly Ser Ala Gln Lys Ile Gln Leu Val Asn
85 90 95
Thr Asn Gly Ser Trp His Ile Asn Arg Thr Ala Leu Asn Cys Asn Asp
100 105 110
Ser Leu Gln Thr Gly Phe Phe Ala Ala Leu Phe Tyr Lys His Lys Phe
115 120 125

Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys Arg Ser Ile Asp
 130 135 140
 Lys Phe Ala Gln Gly Trp Gly Pro Leu Thr Tyr Thr Glu Pro Asn Ser
 145 150 155 160
 Ser Asp Gln Arg Pro Tyr Cys Trp His Tyr Ala Pro Arg Pro Cys Gly
 165 170 175
 Ile Val Pro Ala Ser Gln Val Cys Gly Pro Val Tyr Cys Phe Thr Pro
 180 185 190
 Ser Pro Val Val Val Gly Thr Thr Asp Arg Phe Gly Val Pro Thr Tyr
 195 200 205
 Asn Trp Gly Ala Asn Asp Ser Asp Val Leu Ile Leu Asn Asn Thr Arg
 210 215 220
 Pro Pro Arg Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Gly Thr Gly
 225 230 235 240
 Phe Thr Lys Thr Cys Gly Gly Pro Pro Cys Asn Ile Gly Gly Ala Gly
 245 250 255
 Asn Asn Thr Leu Thr Cys Pro Thr Asp Cys Phe Arg Lys His Pro Glu
 260 265 270
 Ala Thr Tyr Ala Arg Cys Gly Ser Gly Pro Trp Leu Thr Pro Arg Cys
 275 280 285
 Met Val His Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Val Asn
 290 295 300
 Phe Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly Val Glu His Arg
 305 310 315 320
 Phe Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu
 325 330 335
 Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu Ser Thr Thr Glu
 340 345 350
 Trp Gln Ile Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala Leu Ser Thr
 355 360 365
 Gly Leu Ile His Leu His Gln Asn Ile Val Asp Val Gln Tyr Leu Tyr
 370 375 380
 Gly Val Gly Ser Ala Val Val Ser Leu Val Ile Lys Trp Glu Tyr Val
 385 390 395 400
 Leu Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Ile Cys Ala Cys Leu
 405 410 415
 Trp Met Met Leu Leu Ile Ala Gln Ala Glu Ala Ala Leu Glu Asn Leu
 420 425 430
 Val Val Leu Asn Ala Ala Ala Val Ala Gly Ala His Gly Thr Leu Ser
 435 440 445
 Phe Leu Val Phe Phe Cys Ala Ala Trp Tyr Ile Lys Gly Arg Leu Val
 450 455 460
 Pro Gly Ala Ala Tyr Ala Phe Tyr Gly Val Trp Pro Leu Leu Leu Leu
 465 470 475 480
 Leu Leu Ala Leu Pro Pro Arg Ala Tyr Ala
 485 490

(2) INFORMATION FOR SEQ ID NO: 37:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1021 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 2..1018

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
 (B) LOCATION: 2..1015

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

| | |
|---|-----|
| G ATC CCA CAA GCT GTC GTG GAC ATG GTG GCG GGG GCC CAT TGG GGA | 46 |
| Ile Pro Gln Ala Val Val Asp Met Val Ala Gly Ala His Trp Gly | |
| 1 5 10 15 | |
| GTC CTG GCG GGC CTC GCC TAC TAT TCC ATG GTG GGG AAC TGG GCT AAG | 94 |
| Val Leu Ala Gly Leu Ala Tyr Tyr Ser Met Val Gly Asn Trp Ala Lys | |
| 20 25 30 | |
| GTT TTG GTT GTG ATG CTA CTC TTT GCC GGC GTC GAC GGG CAT ACC CGC | 142 |
| Val Leu Val Val Met Leu Leu Phe Ala Gly Val Asp Gly His Thr Arg | |
| 35 40 45 | |
| GTG TCA GGA GGG GCA GCA GCC TCC GAT ACC AGG GGC CTT GTG TCC CTC | 190 |
| Val Ser Gly Gly Ala Ala Ala Ser Asp Thr Arg Gly Leu Val Ser Leu | |
| 50 55 60 | |
| TTT AGC CCC GGG TCG GCT CAG AAA ATC CAG CTC GTA AAC ACC AAC GGC | 238 |
| Phe Ser Pro Gly Ser Ala Gln Lys Ile Gln Leu Val Asn Thr Asn Gly | |
| 65 70 75 | |
| AGT TGG CAC ATC AAC AGG ACT GCC CTG AAC TGC AAC GAC TCC CTC CAA | 286 |
| Ser Trp His Ile Asn Arg Thr Ala Leu Asn Cys Asn Asp Ser Leu Gln | |
| 80 85 90 95 | |
| ACA GGG TTC TTT GCC GCA CTA TTC TAC AAA CAC AAA TTC AAC TCG TCT | 334 |
| Thr Gly Phe Phe Ala Ala Leu Phe Tyr Lys His Lys Phe Asn Ser Ser | |
| 100 105 110 | |
| GGA TGC CCA GAG CGC TTG GCC AGC TGT CGC TCC ATC GAC AAG TTC GCT | 382 |
| Gly Cys Pro Glu Arg Leu Ala Ser Cys Arg Ser Ile Asp Lys Phe Ala | |
| 115 120 125 | |
| CAG GGG TGG GGT CCC CTC ACT TAC ACT GAG CCT AAC AGC TCG GAC CAG | 430 |
| Gln Gly Trp Gly Pro Leu Thr Tyr Thr Glu Pro Asn Ser Ser Asp Gln | |
| 130 135 140 | |
| AGG CCC TAC TGC TGG CAC TAC GCG CCT CGA CCG TGT GGT ATT GTA CCC | 478 |
| Arg Pro Tyr Cys Trp His Tyr Ala Pro Arg Pro Cys Gly Ile Val Pro | |
| 145 150 155 | |
| GCG TCT CAG GTG TGC GGT CCA GTG TAT TGC TTC ACC CCG AGC CCT GTT | 526 |
| Ala Ser Gln Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser Pro Val | |
| 160 165 170 175 | |

| | |
|---|------|
| GTG GTG GGG ACG ACC GAT CGG TTT GGT GTC CCC ACG TAT AAC TGG GGG Val Val Gly Thr Thr Asp Arg Phe Gly Val Pro Thr Tyr Asn Trp Gly 180 185 190 | 574 |
| GCG AAC GAC TCG GAT GTG CTG ATT CTC AAC AAC ACG CGG CCG CCG CGA Ala Asn Asp Ser Asp Val Leu Ile Leu Asn Asn Thr Arg Pro Pro Arg 195 200 205 | 622 |
| GGC AAC TGG TTC GGC TGT ACA TGG ATG AAT GGC ACT GGG TTC ACC AAG Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Gly Thr Gly Phe Thr Lys 210 215 220 | 670 |
| ACG TGT GGG GGC CCC CCG TGC AAC ATC GGG GGG GCC GGC AAC AAC ACC Thr Cys Gly Gly Pro Pro Cys Asn Ile Gly Gly Ala Gly Asn Asn Thr 225 230 235 | 718 |
| TTG ACC TGC CCC ACT GAC TGT TTT CGG AAG CAC CCC GAG GCC ACC TAC Leu Thr Cys Pro Thr Asp Cys Phe Arg Lys His Pro Glu Ala Thr Tyr 240 245 250 255 | 766 |
| GCC AGA TGC GGT TCT GGG CCC TGG CTG ACA CCT AGG TGT ATG GTT CAT Ala Arg Cys Gly Ser Gly Pro Trp Leu Thr Pro Arg Cys Met Val His 260 265 270 | 814 |
| TAC CCA TAT AGG CTC TGG CAC TAC CCC TGC ACT GTC AAC TTC ACC ATC Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Val Asn Phe Thr Ile 275 280 285 | 862 |
| TTC AAG GTT AGG ATG TAC GTG GGG GGC GTG GAG CAC AGG TTC GAA GCC Phe Lys Val Arg Met Tyr Val Gly Gly Val Glu His Arg Phe Glu Ala 290 295 300 | 910 |
| GCA TGC AAT TGG ACT CGA GGA GAG CGT TGT GAC TTG GAG GAC AGG GAT Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu Asp Arg Asp 305 310 315 | 958 |
| AGA TCA GAG CTT AGC CCG CTG CTG CTG TCT ACA ACA GAG TGG CAG AGT Arg Ser Glu Leu Ser Pro Leu Leu Leu Ser Thr Thr Glu Trp Gln Ser 320 325 330 335 | 1006 |
| GGC AGA GCT TAATTA Gly Arg Ala | 1021 |

(2) INFORMATION FOR SEQ ID NO: 38:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 338 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

Ile Pro Gln Ala Val Val Asp Met Val Ala Gly Ala His Trp Gly Val
1 5 10 15
 Leu Ala Gly Leu Ala Tyr Tyr Ser Met Val Gly Asn Trp Ala Lys Val
20 25 30
 Leu Val Val Met Leu Leu Phe Ala Gly Val Asp Gly His Thr Arg Val
35 40 45
 Ser Gly Gly Ala Ala Ala Ser Asp Thr Arg Gly Leu Val Ser Leu Phe
50 55 60
 Ser Pro Gly Ser Ala Gln Lys Ile Gln Leu Val Asn Thr Asn Gly Ser

| 65 | | 70 | | 75 | | 80 | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Trp | His | Ile | Asn | Arg | Thr | Ala | Leu | Asn | Cys | Asn | Asp | Ser | Leu | Gln | Thr |
| | | | 85 | | | | | | 90 | | | | | 95 | |
| Gly | Phe | Phe | Ala | Ala | Leu | Phe | Tyr | Lys | His | Lys | Phe | Asn | Ser | Ser | Gly |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Cys | Pro | Glu | Arg | Leu | Ala | Ser | Cys | Arg | Ser | Ile | Asp | Lys | Phe | Ala | Gln |
| | | 115 | | | | | 120 | | | | | 125 | | | |
| Gly | Trp | Gly | Pro | Leu | Thr | Tyr | Thr | Glu | Pro | Asn | Ser | Ser | Asp | Gln | Arg |
| | 130 | | | | | 135 | | | | | 140 | | | | |
| Pro | Tyr | Cys | Trp | His | Tyr | Ala | Pro | Arg | Pro | Cys | Gly | Ile | Val | Pro | Ala |
| 145 | | | | | 150 | | | | | 155 | | | | 160 | |
| Ser | Gln | Val | Cys | Gly | Pro | Val | Tyr | Cys | Phe | Thr | Pro | Ser | Pro | Val | Val |
| | | | 165 | | | | | | 170 | | | | | 175 | |
| Val | Gly | Thr | Thr | Asp | Arg | Phe | Gly | Val | Pro | Thr | Tyr | Asn | Trp | Gly | Ala |
| | | | 180 | | | | | 185 | | | | | 190 | | |
| Asn | Asp | Ser | Asp | Val | Leu | Ile | Leu | Asn | Asn | Thr | Arg | Pro | Pro | Arg | Gly |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| Asn | Trp | Phe | Gly | Cys | Thr | Trp | Met | Asn | Gly | Thr | Gly | Phe | Thr | Lys | Thr |
| | 210 | | | | | 215 | | | | | 220 | | | | |
| Cys | Gly | Gly | Pro | Pro | Cys | Asn | Ile | Gly | Gly | Ala | Gly | Asn | Asn | Thr | Leu |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| Thr | Cys | Pro | Thr | Asp | Cys | Phe | Arg | Lys | His | Pro | Glu | Ala | Thr | Tyr | Ala |
| | | | 245 | | | | | | 250 | | | | | 255 | |
| Arg | Cys | Gly | Ser | Gly | Pro | Trp | Leu | Thr | Pro | Arg | Cys | Met | Val | His | Tyr |
| | | | 260 | | | | | 265 | | | | | 270 | | |
| Pro | Tyr | Arg | Leu | Trp | His | Tyr | Pro | Cys | Thr | Val | Asn | Phe | Thr | Ile | Phe |
| | 275 | | | | | | 280 | | | | | 285 | | | |
| Lys | Val | Arg | Met | Tyr | Val | Gly | Gly | Val | Glu | His | Arg | Phe | Glu | Ala | Ala |
| | 290 | | | | | 295 | | | | | 300 | | | | |
| Cys | Asn | Trp | Thr | Arg | Gly | Glu | Arg | Cys | Asp | Leu | Glu | Asp | Arg | Asp | Arg |
| 305 | | | | | 310 | | | | | 315 | | | | 320 | |
| Ser | Glu | Leu | Ser | Pro | Leu | Leu | Leu | Ser | Thr | Thr | Glu | Trp | Gln | Ser | Gly |
| | | | 325 | | | | | | 330 | | | | | 335 | |
| Arg | Ala | | | | | | | | | | | | | | |

(2) INFORMATION FOR SEQ ID NO: 39:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1034 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 2..1032

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
(B) LOCATION: 2..1029

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

| | |
|---|-----|
| G ATC CCA CAA GCT GTC GTG GAC ATG GTG GCG GGG GCC CAT TGG GGA Ile Pro Gln Ala Val Val Asp Met Val Ala Gly Ala His Trp Gly 1 5 10 15 | 46 |
| GTC CTG GCG GGC CTC GCC TAC TAT TCC ATG GTG GGG AAC TGG GCT AAG Val Leu Ala Gly Leu Ala Tyr Tyr Ser Met Val Gly Asn Trp Ala Lys 20 25 30 | 94 |
| GTT TTG GTT GTG ATG CTA CTC TTT GCC GGC GTC GAC GGG CAT ACC CGC Val Leu Val Val Met Leu Leu Phe Ala Gly Val Asp Gly His Thr Arg 35 40 45 | 142 |
| GTG TCA GGA GGG GCA GCA GCC TCC GAT ACC AGG GGC CTT GTG TCC CTC Val Ser Gly Gly Ala Ala Ala Ser Asp Thr Arg Gly Leu Val Ser Leu 50 55 60 | 190 |
| TTT AGC CCC GGG TCG GCT CAG AAA ATC CAG CTC GTA AAC ACC AAC GGC Phe Ser Pro Gly Ser Ala Gln Lys Ile Gln Leu Val Asn Thr Asn Gly 65 70 75 | 238 |
| AGT TGG CAC ATC AAC AGG ACT GCC CTG AAC TGC AAC GAC TCC CTC CAA Ser Trp His Ile Asn Arg Thr Ala Leu Asn Cys Asn Asp Ser Leu Gln 80 85 90 95 | 286 |
| ACA GGG TTC TTT GCC GCA CTA TTC TAC AAA CAC AAA TTC AAC TCG TCT Thr Gly Phe Phe Ala Ala Leu Phe Tyr Lys His Lys Phe Asn Ser Ser 100 105 110 | 334 |
| GGA TGC CCA GAG CGC TTG GCC AGC TGT CGC TCC ATC GAC AAG TTC GCT Gly Cys Pro Glu Arg Leu Ala Ser Cys Arg Ser Ile Asp Lys Phe Ala 115 120 125 | 382 |
| CAG GGG TGG GGT CCC CTC ACT TAC ACT GAG CCT AAC AGC TCG GAC CAG Gln Gly Trp Gly Pro Leu Thr Tyr Thr Glu Pro Asn Ser Ser Asp Gln 130 135 140 | 430 |
| AGG CCC TAC TGC TGG CAC TAC GCG CCT CGA CCG TGT GGT ATT GTA CCC Arg Pro Tyr Cys Trp His Tyr Ala Pro Arg Pro Cys Gly Ile Val Pro 145 150 155 | 478 |
| GCG TCT CAG GTG TGC GGT CCA GTG TAT TGC TTC ACC CCG AGC CCT GTT Ala Ser Gln Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser Pro Val 160 165 170 175 | 526 |
| GTG GTG GGG ACG ACC GAT CGG TTT GGT GTC CCC ACG TAT AAC TGG GGG Val Val Gly Thr Thr Asp Arg Phe Gly Val Pro Thr Tyr Asn Trp Gly 180 185 190 | 574 |
| GCG AAC GAC TCG GAT GTG CTG ATT CTC AAC AAC ACG CCG CCG CCG CGA Ala Asn Asp Ser Asp Val Leu Ile Leu Asn Asn Thr Arg Pro Pro Arg 195 200 205 | 622 |
| GGC AAC TGG TTC GGC TGT ACA TGG ATG AAT GGC ACT GGG TTC ACC AAG Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Gly Thr Gly Phe Thr Lys 210 215 220 | 670 |
| ACG TGT GGG GGC CCC CCG TGC AAC ATC GGG GGG GCC GGC AAC AAC ACC Thr Cys Gly Gly Pro Pro Cys Asn Ile Gly Gly Ala Gly Asn Asn Thr 225 230 235 | 718 |

| | |
|---|------|
| TTG ACC TGC CCC ACT GAC TGT TTT CGG AAG CAC CCC GAG GCC ACC TAC Leu Thr Cys Pro Thr Asp Cys Phe Arg Lys His Pro Glu Ala Thr Tyr 240 245 250 255 | 766 |
| GCC AGA TGC GGT TCT GGG CCC TGG CTG ACA CCT AGG TGT ATG GTT CAT Ala Arg Cys Gly Ser Gly Pro Trp Leu Thr Pro Arg Cys Met Val His 260 265 270 | 814 |
| TAC CCA TAT AGG CTC TGG CAC TAC CCC TGC ACT GTC AAC TTC ACC ATC Tyr Pro Tyr 275 Leu Trp His Tyr 280 Cys Thr Val Asn Phe Thr Ile 285 | 862 |
| TTC AAG GTT AGG ATG TAC GTG GGG GGC GTG GAG CAC AGG TTC GAA GCC Phe Lys Val Arg Met Tyr Val Gly Val Glu His Arg Phe Glu Ala 290 295 300 | 910 |
| GCA TGC AAT TGG ACT CGA GGA GAG CGT TGT GAC TTG GAG GAC AGG GAT Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu Asp Arg Asp 305 310 315 | 958 |
| AGA TCA GAG CTT AGC CCG CTG CTG CTG TCT ACA ACA GGT GAT CGA GGG Arg Ser Glu Leu Ser Pro Leu Leu Leu Ser Thr Thr Gly Asp Arg Gly 320 325 330 335 | 1006 |
| CAG ACA CCA TCA CCA CCA TCA CTA AT AG Gln Thr Pro Ser Pro Pro Ser Leu 340 | 1034 |

(2) INFORMATION FOR SEQ ID NO: 40:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 343 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

| |
|--|
| Ile Pro Gln Ala Val Val Asp Met Val Ala Gly Ala His Trp Gly Val 1 5 10 15 |
| Leu Ala Gly Leu Ala Tyr Tyr Ser Met Val Gly Asn Trp Ala Lys Val 20 25 30 |
| Leu Val Val Met Leu Leu Phe Ala Gly Val Asp Gly His Thr Arg Val 35 40 45 |
| Ser Gly Gly Ala Ala Ala Ser Asp Thr Arg Gly Leu Val Ser Leu Phe 50 55 60 |
| Ser Pro Gly Ser Ala Gln Lys Ile Gln Leu Val Asn Thr Asn Gly Ser 65 70 75 80 |
| Trp His Ile Asn Arg Thr Ala Leu Asn Cys Asn Asp Ser Leu Gln Thr 85 90 95 |
| Gly Phe Phe Ala Ala Leu Phe Tyr Lys His Lys Phe Asn Ser Ser Gly 100 105 110 |
| Cys Pro Glu Arg Leu Ala Ser Cys Arg Ser Ile Asp Lys Phe Ala Gln 115 120 125 |
| Gly Trp Gly Pro Leu Thr Tyr Thr Glu Pro Asn Ser Ser Asp Gln Arg 130 135 140 |
| Pro Tyr Cys Trp His Tyr Ala Pro Arg Pro Cys Gly Ile Val Pro Ala 145 150 155 160 |

- 117 -

Ser Gln Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser Pro Val Val
165 170 175
Val Gly Thr Thr Asp Arg Phe Gly Val Pro Thr Tyr Asn Trp Gly Ala
180 185 190
Asn Asp Ser Asp Val Leu Ile Leu Asn Asn Thr Arg Pro Pro Arg Gly
195 200 205
Asn Trp Phe Gly Cys Thr Trp Met Asn Gly Thr Gly Phe Thr Lys Thr
210 215 220
Cys Gly Gly Pro Pro Cys Asn Ile Gly Gly Ala Gly Asn Asn Thr Leu
225 230 235 240
Thr Cys Pro Thr Asp Cys Phe Arg Lys His Pro Glu Ala Thr Tyr Ala
245 250 255
Arg Cys Gly Ser Gly Pro Trp Leu Thr Pro Arg Cys Met Val His Tyr
260 265 270
Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Val Asn Phe Thr Ile Phe
275 280 285
Lys Val Arg Met Tyr Val Gly Gly Val Glu His Arg Phe Glu Ala Ala
290 295 300
Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu Asp Arg Asp Arg
305 310 315 320
Ser Glu Leu Ser Pro Leu Leu Leu Ser Thr Thr Gly Asp Arg Gly Gln
325 330 335
Thr Pro Ser Pro Pro Ser Leu
340

(2) INFORMATION FOR SEQ ID NO: 41:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 945 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

- (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..942

- (ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..939

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| ATG | GTG | GGG | AAC | TGG | GCT | AAG | GTT | TTG | GTT | GTG | ATG | CTA | CTC | TTT | GCC | 48 |
| Met | Val | Gly | Asn | Trp | Ala | Lys | Val | Leu | Val | Met | Leu | Leu | Phe | Ala | | |
| 1 | | | | 5 | | | | 10 | | | | | | 15 | | |
| GGC | GTC | GAC | GGG | CAT | ACC | CGC | GTG | TCA | GGA | GGG | GCA | GCA | GCC | TCC | GAT | 96 |
| Gly | Val | Asp | Gly | His | Thr | Arg | Val | Ser | Gly | Gly | Ala | Ala | Ala | Ser | Asp | |

| 20 | 25 | 30 | |
|---|----|----|-----|
| ACC AGG GGC CTT GTG TCC CTC TTT AGC CCC GGG TCG GCT CAG AAA ATC Thr Arg Gly Leu Val Ser Leu Phe Ser Pro Gly Ser Ala Gln Lys Ile 35 40 45 | | | 144 |
| CAG CTC GTA AAC ACC AAC GGC AGT TGG CAC ATC AAC AGG ACT GCC CTG Gln Leu Val Asn Thr Asn Gly Ser Trp His Ile Asn Arg Thr Ala Leu 50 55 60 | | | 192 |
| AAC TGC AAC GAC TCC CTC CAA ACA GGG TTC TTT GCC GCA CTA TTC TAC Asn Cys Asn Asp Ser Leu Gln Thr Gly Phe Phe Ala Ala Leu Phe Tyr 65 70 75 80 | | | 240 |
| AAA CAC AAA TTC AAC TCG TCT GGA TGC CCA GAG CGC TTG GCC AGC TGT Lys His Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys 85 90 95 | | | 288 |
| CGC TCC ATC GAC AAG TTC GCT CAG GGG TGG GGT CCC CTC ACT TAC ACT Arg Ser Ile Asp Lys Phe Ala Gln Gly Trp Gly Pro Leu Thr Tyr Thr 100 105 110 | | | 336 |
| GAG CCT AAC AGC TCG GAC CAG AGG CCC TAC TGC TGG CAC TAC GCG CCT Glu Pro Asn Ser Ser Asp Gln Arg Pro Tyr Cys Trp His Tyr Ala Pro 115 120 125 | | | 384 |
| CGA CCG TGT GGT ATT GTA CCC GCG TCT CAG GTG TGC GGT CCA GTG TAT Arg Pro Cys Gly Ile Val Pro Ala Ser Gln Val Cys Gly Pro Val Tyr 130 135 140 | | | 432 |
| TGC TTC ACC CCG AGC CCT GTT GTG GTG GGG ACG ACC GAT CGG TTT GGT Cys Phe Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Phe Gly 145 150 155 160 | | | 480 |
| GTC CCC ACG TAT AAC TGG GGG GCG AAC GAC TCG GAT GTG CTG ATT CTC Val Pro Thr Tyr Asn Trp Gly Ala Asn Asp Ser Asp Val Leu Ile Leu 165 170 175 | | | 528 |
| AAC AAC ACG CGG CCG CCG CGA GGC AAC TGG TTC GGC TGT ACA TGG ATG Asn Asn Thr Arg Pro Pro Arg Gly Asn Trp Phe Gly Cys Thr Trp Met 180 185 190 | | | 576 |
| AAT GGC ACT GGG TTC ACC AAG ACG TGT GGG GGC CCC CCG TGC AAC ATC Asn Gly Thr Gly Phe Thr Lys Thr Cys Gly Gly Pro Pro Cys Asn Ile 195 200 205 | | | 624 |
| GGG GGG GCC GGC AAC AAC ACC TTG ACC TGC CCC ACT GAC TGT TTT CGG Gly Gly Ala Gly Asn Asn Thr Leu Thr Cys Pro Thr Asp Cys Phe Arg 210 215 220 | | | 672 |
| AAG CAC CCC GAG GCC ACC TAC GCC AGA TGC GGT TCT GGG CCC TGG CTG Lys His Pro Glu Ala Thr Tyr Ala Arg Cys Gly Ser Gly Pro Trp Leu 225 230 235 240 | | | 720 |
| ACA CCT AGG TGT ATG GTT CAT TAC CCA TAT AGG CTC TGG CAC TAC CCC Thr Pro Arg Cys Met Val His Tyr Pro Tyr Arg Leu Trp His Tyr Pro 245 250 255 | | | 768 |
| TGC ACT GTC AAC TTC ACC ATC TTC AAG GTT AGG ATG TAC GTG GGG GGC Cys Thr Val Asn Phe Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly 260 265 270 | | | 816 |
| GTG GAG CAC AGG TTC GAA GCC GCA TGC AAT TGG ACT CGA GGA GAG CGT Val Glu His Arg Phe Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg 275 280 285 | | | 864 |
| TGT GAC TTG GAG GAC AGG GAT AGA TCA GAG CTT AGC CCG CTG CTG CTG Cys Asp Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu 290 295 300 | | | 912 |

TCT ACA ACA GAG TGG CAG AGC TTA ATT AAT TAG
Ser Thr Thr Glu Trp Gln Ser Leu Ile Asn
305 310

945

(2) INFORMATION FOR SEQ ID NO: 42:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 314 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

Met Val Gly Asn Trp Ala Lys Val Leu Val Val Met Leu Leu Phe Ala
1 5 10 15
Gly Val Asp Gly His Thr Arg Val Ser Gly Gly Ala Ala Ala Ser Asp
20 25 30
Thr Arg Gly Leu Val Ser Leu Phe Ser Pro Gly Ser Ala Gln Lys Ile
35 40 45
Gln Leu Val Asn Thr Asn Gly Ser Trp His Ile Asn Arg Thr Ala Leu
50 55 60
Asn Cys Asn Asp Ser Leu Gln Thr Gly Phe Phe Ala Ala Leu Phe Tyr
65 70 75 80
Lys His Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys
85 90 95
Arg Ser Ile Asp Lys Phe Ala Gln Gly Trp Gly Pro Leu Thr Tyr Thr
100 105 110
Glu Pro Asn Ser Ser Asp Gln Arg Pro Tyr Cys Trp His Tyr Ala Pro
115 120 125
Arg Pro Cys Gly Ile Val Pro Ala Ser Gln Val Cys Gly Pro Val Tyr
130 135 140
Cys Phe Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Phe Gly
145 150 155 160
Val Pro Thr Tyr Asn Trp Gly Ala Asn Asp Ser Asp Val Leu Ile Leu
165 170 175
Asn Asn Thr Arg Pro Pro Arg Gly Asn Trp Phe Gly Cys Thr Trp Met
180 185 190
Asn Gly Thr Gly Phe Thr Lys Thr Cys Gly Gly Pro Pro Cys Asn Ile
195 200 205
Gly Gly Ala Gly Asn Asn Thr Leu Thr Cys Pro Thr Asp Cys Phe Arg
210 215 220
Lys His Pro Glu Ala Thr Tyr Ala Arg Cys Gly Ser Gly Pro Trp Leu
225 230 235 240
Thr Pro Arg Cys Met Val His Tyr Pro Tyr Arg Leu Trp His Tyr Pro
245 250 255
Cys Thr Val Asn Phe Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly
260 265 270
Val Glu His Arg Phe Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg
275 280 285

Cys Asp Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu
290 295 300

Ser Thr Thr Glu Trp Gln Ser Leu Ile Asn
305 310

(2) INFORMATION FOR SEQ ID NO: 43:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 961 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

- (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..958

- (ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..955

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

| | |
|---|-----|
| ATG GTG GGG AAC TGG GCT AAG GTT TTG GTT GTG ATG CTA CTC TTT GCC | 48 |
| Met Val Gly Asn Trp Ala Lys Val Leu Val Val Met Leu Leu Phe Ala | |
| 1 5 10 15 | |
| GGC GTC GAC GGG CAT ACC CGC GTG TCA GGA GGG GCA GCA GCC TCC GAT | 96 |
| Gly Val Asp Gly His Thr Arg Val Ser Gly Gly Ala Ala Ala Ser Asp | |
| 20 25 30 | |
| ACC AGG GGC CTT GTG TCC CTC TTT AGC CCC GGG TCG GCT CAG AAA ATC | 144 |
| Thr Arg Gly Leu Val Ser Leu Phe Ser Pro Gly Ser Ala Gln Lys Ile | |
| 35 40 45 | |
| CAG CTC GTA AAC ACC AAC GGC AGT TGG CAC ATC AAC AGG ACT GCC CTG | 192 |
| Gln Leu Val Asn Thr Asn Gly Ser Trp His Ile Asn Arg Thr Ala Leu | |
| 50 55 60 | |
| AAC TGC AAC GAC TCC CTC CAA ACA GGG TTC TTT GCC GCA CTA TTC TAC | 240 |
| Asn Cys Asn Asp Ser Leu Gln Thr Gly Phe Phe Ala Ala Leu Phe Tyr | |
| 65 70 75 80 | |
| AAA CAC AAA TTC AAC TCG TCT GGA TGC CCA GAG CGC TTG GCC AGC TGT | 288 |
| Lys His Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys | |
| 85 90 95 | |
| CGC TCC ATC GAC AAG TTC GCT CAG GGG TGG GGT CCC CTC ACT TAC ACT | 336 |
| Arg Ser Ile Asp Lys Phe Ala Gln Gly Trp Gly Pro Leu Thr Tyr Thr | |
| 100 105 110 | |
| GAG CCT AAC AGC TCG GAC CAG AGG CCC TAC TGC TGG CAC TAC GCG CCT | 384 |
| Glu Pro Asn Ser Ser Asp Gln Arg Pro Tyr Cys Trp His Tyr Ala Pro | |
| 115 120 125 | |
| CGA CCG TGT GGT ATT GTA CCC GCG TCT CAG GTG TGC GGT CCA GTG TAT | 432 |
| Arg Pro Cys Gly Ile Val Pro Ala Ser Gln Val Cys Gly Pro Val Tyr | |
| 130 135 140 | |
| TGC TTC ACC CCG AGC CCT GTT GTG GTG GGG ACG ACC GAT CGG TTT GGT | 480 |

| | | | | | | | | | | | | | | | | |
|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|-----|
| Cys 145 | Phe | Thr | Pro | Ser | Pro 150 | Val | Val | Val | Gly | Thr 155 | Thr | Asp | Arg | Phe | Gly 160 | |
| GTC | CCC | ACG | TAT | AAC | TGG | GGG | GCG | AAC | GAC | TCG | GAT | GTG | CTG | ATT | CTC | 528 |
| Val | Pro | Thr | Tyr | Asn 165 | Trp | Gly | Ala | Asn | Asp 170 | Ser | Asp | Val | Leu | Ile 175 | Leu | |
| AAC | AAC | ACG | CGG | CCG | CCG | CGA | GGC | AAC | TGG | TTC | GGC | TGT | ACA | TGG | ATG | 576 |
| Asn | Asn | Thr | Arg 180 | Pro | Pro | Arg | Gly | Asn 185 | Trp | Phe | Gly | Cys | Thr 190 | Trp | Met | |
| AAT | GGC | ACT | GGG | TTC | ACC | AAG | ACG | TGT | GGG | GGC | CCC | CCG | TGC | AAC | ATC | 624 |
| Asn | Gly | Thr 195 | Gly | Phe | Thr | Lys | Thr 200 | Cys | Gly | Gly | Pro | Cys 205 | Pro | Asn | Ile | |
| GGG | GGG | GCC | GGC | AAC | AAC | ACC | TTG | ACC | TGC | CCC | ACT | GAC | TGT | TTT | CGG | 672 |
| Gly | Gly | Ala | Gly | Asn | Asn | Thr 215 | Leu | Thr | Cys | Pro | Thr 220 | Asp | Cys | Phe | Arg | |
| AAG | CAC | CCC | GAG | GCC | ACC | TAC | GCC | AGA | TGC | GGT | TCT | GGG | CCC | TGG | CTG | 720 |
| Lys | His | Pro | Glu | Ala | Thr 230 | Tyr | Ala | Arg | Cys | Gly 235 | Ser | Gly | Pro | Trp | Leu 240 | |
| ACA | CCT | AGG | TGT | ATG | GTT | CAT | TAC | CCA | TAT | AGG | CTC | TGG | CAC | TAC | CCC | 768 |
| Thr | Pro | Arg | Cys | Met 245 | Val | His | Tyr | Pro | Tyr 250 | Arg | Leu | Trp | His | Tyr 255 | Pro | |
| TGC | ACT | GTC | AAC | TTC | ACC | ATC | TTC | AAG | GTT | AGG | ATG | TAC | GTG | GGG | GGC | 816 |
| Cys | Thr | Val | Asn 260 | Phe | Thr | Ile | Phe | Lys 265 | Val | Arg | Met | Tyr 270 | Val | Gly | Gly | |
| GTG | GAG | CAC | AGG | TTC | GAA | GCC | GCA | TGC | AAT | TGG | ACT | CGA | GGA | GAG | CGT | 864 |
| Val | Glu | His 275 | Arg | Phe | Glu | Ala | Ala 280 | Cys | Asn | Trp | Thr | Arg 285 | Gly | Glu | Arg | |
| TGT | GAC | TTG | GAG | GAC | AGG | GAT | AGA | TCA | GAG | CTT | AGC | CCG | CTG | CTG | CTG | 912 |
| Cys | Asp 290 | Leu | Glu | Asp | Arg | Asp 295 | Arg | Ser | Glu | Leu | Ser 300 | Pro | Leu | Leu | Leu | |
| TCT | ACA | ACA | GGT | GAT | CGA | GGG | CAG | ACA | CCA | TCA | CCA | CCA | TCA | CTA | A | 958 |
| Ser | Thr | Thr | Gly | Asp | Arg 310 | Gly | Gln | Thr | Pro | Ser 315 | Pro | Pro | Ser | Leu | | |
| 305 | | | | | | | | | | | | | | | | |
| TAG | | | | | | | | | | | | | | | | 961 |

(2) INFORMATION FOR SEQ ID NO: 44:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 319 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

Met Val Gly Asn Trp Ala Lys Val Leu Val Val Met Leu Leu Phe Ala
 1 5 10 15
 Gly Val Asp Gly His Thr Arg Val Ser Gly Gly Ala Ala Ala Ser Asp
 20 25 30
 Thr Arg Gly Leu Val Ser Leu Phe Ser Pro Gly Ser Ala Gln Lys Ile
 35 40 45
 Gln Leu Val Asn Thr Asn Gly Ser Trp His Ile Asn Arg Thr Ala Leu
 50 55 60

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Asn Cys Asn Asp Ser Leu Gln Thr Gly Phe Phe Ala Ala Leu Phe Tyr
 65              70              75              80
Lys His Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys
              85              90              95
Arg Ser Ile Asp Lys Phe Ala Gln Gly Trp Gly Pro Leu Thr Tyr Thr
              100              105              110
Glu Pro Asn Ser Ser Asp Gln Arg Pro Tyr Cys Trp His Tyr Ala Pro
              115              120              125
Arg Pro Cys Gly Ile Val Pro Ala Ser Gln Val Cys Gly Pro Val Tyr
              130              135              140
Cys Phe Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Phe Gly
145              150              155              160
Val Pro Thr Tyr Asn Trp Gly Ala Asn Asp Ser Asp Val Leu Ile Leu
              165              170              175
Asn Asn Thr Arg Pro Pro Arg Gly Asn Trp Phe Gly Cys Thr Trp Met
              180              185              190
Asn Gly Thr Gly Phe Thr Lys Thr Cys Gly Gly Pro Pro Cys Asn Ile
              195              200              205
Gly Gly Ala Gly Asn Asn Thr Leu Thr Cys Pro Thr Asp Cys Phe Arg
210              215              220
Lys His Pro Glu Ala Thr Tyr Ala Arg Cys Gly Ser Gly Pro Trp Leu
225              230              235              240
Thr Pro Arg Cys Met Val His Tyr Pro Tyr Arg Leu Trp His Tyr Pro
              245              250              255
Cys Thr Val Asn Phe Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly
              260              265              270
Val Glu His Arg Phe Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg
              275              280              285
Cys Asp Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu
290              295              300
Ser Thr Thr Gly Asp Arg Gly Gln Thr Pro Ser Pro Pro Ser Leu
305              310              315

```

(2) INFORMATION FOR SEQ ID NO: 45:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1395 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1392

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide

(B) LOCATION: 1..1389

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ATG | GTG | GCG | GGG | GCC | CAT | TGG | GGA | GTC | CTG | GCG | GGC | CTC | GCC | TAC | TAT | 48 |
| Met | Val | Ala | Gly | Ala | His | Trp | Gly | Val | Leu | Ala | Gly | Leu | Ala | Tyr | Tyr | |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | | |
| TCC | ATG | GTG | GGG | AAC | TGG | GCT | AAG | GTT | TTG | GTT | GTG | ATG | CTA | CTC | TTT | 96 |
| Ser | Met | Val | Gly | Asn | Trp | Ala | Lys | Val | Leu | Val | Val | Met | Leu | Leu | Phe | |
| | | | 20 | | | | | 25 | | | | | 30 | | | |
| GCC | GGC | GTC | GAC | GGG | CAT | ACC | CGC | GTG | TCA | GGA | GGG | GCA | GCA | GCC | TCC | 144 |
| Ala | Gly | Val | Asp | Gly | His | Thr | Arg | Val | Ser | Gly | Gly | Ala | Ala | Ala | Ser | |
| | | 35 | | | | | 40 | | | | | 45 | | | | |
| GAT | ACC | AGG | GGC | CTT | GTG | TCC | CTC | TTT | AGC | CCC | GGG | TCG | GCT | CAG | AAA | 192 |
| Asp | Thr | Arg | Gly | Leu | Val | Ser | Leu | Phe | Ser | Pro | Gly | Ser | Ala | Gln | Lys | |
| | 50 | | | | | 55 | | | | | 60 | | | | | |
| ATC | CAG | CTC | GTA | AAC | ACC | AAC | GGC | AGT | TGG | CAC | ATC | AAC | AGG | ACT | GCC | 240 |
| Ile | Gln | Leu | Val | Asn | Thr | Asn | Gly | Ser | Trp | His | Ile | Asn | Arg | Thr | Ala | |
| 65 | | | | 70 | | | | | | 75 | | | | | 80 | |
| CTG | AAC | TGC | AAC | GAC | TCC | CTC | CAA | ACA | GGG | TTC | TTT | GCC | GCA | CTA | TTC | 288 |
| Leu | Asn | Cys | Asn | Asp | Ser | Leu | Gln | Thr | Gly | Phe | Phe | Ala | Ala | Leu | Phe | |
| | | | | 85 | | | | | 90 | | | | | 95 | | |
| TAC | AAA | CAC | AAA | TTC | AAC | TCG | TCT | GGA | TGC | CCA | GAG | CGC | TTG | GCC | AGC | 336 |
| Tyr | Lys | His | Lys | Phe | Asn | Ser | Ser | Gly | Cys | Pro | Glu | Arg | Leu | Ala | Ser | |
| | | | 100 | | | | | 105 | | | | | 110 | | | |
| TGT | CGC | TCC | ATC | GAC | AAG | TTC | GCT | CAG | GGG | TGG | GGT | CCC | CTC | ACT | TAC | 384 |
| Cys | Arg | Ser | Ile | Asp | Lys | Phe | Ala | Gln | Gly | Trp | Gly | Pro | Leu | Thr | Tyr | |
| | | 115 | | | | 120 | | | | | 125 | | | | | |
| ACT | GAG | CCT | AAC | AGC | TCG | GAC | CAG | AGG | CCC | TAC | TGC | TGG | CAC | TAC | GCG | 432 |
| Thr | Glu | Pro | Asn | Ser | Ser | Asp | Gln | Arg | Pro | Tyr | Cys | Trp | His | Tyr | Ala | |
| | 130 | | | | | 135 | | | | | 140 | | | | | |
| CCT | CGA | CCG | TGT | GGT | ATT | GTA | CCC | GCG | TCT | CAG | GTG | TGC | GGT | CCA | GTG | 480 |
| Pro | Arg | Pro | Cys | Gly | Ile | Val | Pro | Ala | Ser | Gln | Val | Cys | Gly | Pro | Val | |
| 145 | | | | | 150 | | | | | 155 | | | | | 160 | |
| TAT | TGC | TTC | ACC | CCG | AGC | CCT | GTT | GTG | GTG | GGG | ACG | ACC | GAT | CGG | TTT | 528 |
| Tyr | Cys | Phe | Thr | Pro | Ser | Pro | Val | Val | Val | Gly | Thr | Thr | Asp | Arg | Phe | |
| | | | | 165 | | | | 170 | | | | | | 175 | | |
| GGT | GTC | CCC | ACG | TAT | AAC | TGG | GGG | GCG | AAC | GAC | TCG | GAT | GTG | CTG | ATT | 576 |
| Gly | Val | Pro | Thr | Tyr | Asn | Trp | Gly | Ala | Asn | Asp | Ser | Asp | Val | Leu | Ile | |
| | | | 180 | | | | | 185 | | | | | 190 | | | |
| CTC | AAC | AAC | ACG | CGG | CCG | CCG | CGA | GCG | AAC | TGG | TTC | GGC | TGT | ACA | TGG | 624 |
| Leu | Asn | Asn | Thr | Arg | Pro | Pro | Arg | Gly | Asn | Trp | Phe | Gly | Cys | Thr | Trp | |
| | | 195 | | | | | 200 | | | | | 205 | | | | |
| ATG | AAT | GGC | ACT | GGG | TTC | ACC | AAG | ACG | TGT | GGG | GGC | CCC | CCG | TGC | AAC | 672 |
| Met | Asn | Gly | Thr | Gly | Phe | Thr | Lys | Thr | Cys | Gly | Gly | Pro | Pro | Cys | Asn | |
| | 210 | | | | | 215 | | | | | 220 | | | | | |
| ATC | GGG | GGG | GCC | GGC | AAC | AAC | ACC | TTG | ACC | TGC | CCC | ACT | GAC | TGT | TTT | 720 |
| Ile | Gly | Gly | Ala | Gly | Asn | Asn | Thr | Leu | Thr | Cys | Pro | Thr | Asp | Cys | Phe | |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 | |
| CGG | AAG | CAC | CCC | GAG | GCC | ACC | TAC | GCC | AGA | TGC | GGT | TCT | GGG | CCC | TGG | 758 |
| Arg | Lys | His | Pro | Glu | Ala | Thr | Tyr | Ala | Arg | Cys | Gly | Ser | Gly | Pro | Trp | |
| | | | | 245 | | | | | 250 | | | | | 255 | | |

| | |
|---|------|
| CTG ACA CCT AGG TGT ATG GTT CAT TAC CCA TAT AGG CTC TGG CAC TAC Leu Thr Pro Arg Cys Met Val His Tyr Pro Tyr Arg Leu Trp His Tyr 260 265 | 816 |
| CCC TGC ACT GTC AAC TTC ACC ATC TTC AAG GTT AGG ATG TAC GTG GGG Pro Cys Thr Val Asn Phe Thr Ile Phe Lys Val Arg Met Tyr Val Gly 275 280 285 | 864 |
| GGC GTG GAG CAC AGG TTC GAA GCC GCA TGC AAT TGG ACT CGA GGA GAG Gly Val Glu His Arg Phe Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu 290 295 300 | 912 |
| CGT TGT GAC TTG GAG GAC AGG GAT AGA TCA GAG CTT AGC CCG CTG CTG Arg Cys Asp Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu 305 310 315 320 | 960 |
| CTG TCT ACA ACA GAG TGG CAG ATA CTG CCC TGT TCC TTC ACC ACC CTG Leu Ser Thr Thr Glu Trp Gln Ile Leu Pro Cys Ser Phe Thr Thr Leu 325 330 335 | 1008 |
| CCG GCC CTA TCC ACC GGC CTG ATC CAC CTC CAT CAG AAC ATC GTG GAC Pro Ala Leu Ser Thr Gly Leu Ile His Leu His Gln Asn Ile Val Asp 340 345 350 | 1056 |
| GTG CAA TAC CTG TAC GGT GTA GGG TCG GCG GTT GTC TCC CTT GTC ATC Val Gln Tyr Leu Tyr Gly Val Gly Ser Ala Val Val Ser Leu Val Ile 355 360 365 | 1104 |
| AAA TGG GAG TAT GTC CTG TTG CTC TTC CTT CTC CTG GCA GAC GCG CGC Lys Trp Glu Tyr Val Leu Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg 370 375 380 | 1152 |
| ATC TGC GCC TGC TTA TGG ATG ATG CTG CTG ATA GCT CAA GCT GAG GCC Ile Cys Ala Cys Leu Trp Met Met Leu Leu Ile Ala Gln Ala Glu Ala 385 390 395 400 | 1200 |
| GCC TTA GAG AAC CTG GTG GTC CTC AAT GCG GCG GCC GTG GCC GGG GCG Ala Leu Glu Asn Leu Val Val Leu Asn Ala Ala Ala Val Ala Gly Ala 405 410 415 | 1248 |
| CAT GGC ACT CTT TCC TTC CTT GTG TTC TTC TGT GCT GCC TGG TAC ATC His Gly Thr Leu Ser Phe Leu Val Phe Phe Cys Ala Ala Trp Tyr Ile 420 425 430 | 1296 |
| AAG GGC AGG CTG GTC CCT GGT GCG GCA TAC GCC TTC TAT GGC GTG TGG Lys Gly Arg Leu Val Pro Gly Ala Ala Tyr Ala Phe Tyr Gly Val Trp 435 440 445 | 1344 |
| CCG CTG CTC CTG CTT CTG CTG GCC TTA CCA CCA CGA GCT TAT GCC TAGTAA Pro Leu Leu Leu Leu Leu Leu Ala Leu Pro Pro Arg Ala Tyr Ala 450 455 460 | 1395 |

(2) INFORMATION FOR SEQ ID NO: 46:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 463 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

| |
|--|
| Met Val Ala Gly Ala His Trp Gly Val Leu Ala Gly Leu Ala Tyr Tyr 1 5 10 15 |
| Ser Met Val Gly Asn Trp Ala Lys Val Leu Val Val Met Leu Leu Phe 20 25 30 |

Ala Gly Val Asp Gly His Thr Arg Val Ser Gly Gly Ala Ala Ala Ser
35 40 45

Asp Thr Arg Gly Leu Val Ser Leu Phe Ser Pro Gly Ser Ala Gln Lys
50 55 60

Ile Gln Leu Val Asn Thr Asn Gly Ser Trp His Ile Asn Arg Thr Ala
65 70 75 80

Leu Asn Cys Asn Asp Ser Leu Gln Thr Gly Phe Phe Ala Ala Leu Phe
85 90 95

Tyr Lys His Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser
100 105 110

Cys Arg Ser Ile Asp Lys Phe Ala Gln Gly Trp Gly Pro Leu Thr Tyr
115 120 125

Thr Glu Pro Asn Ser Ser Asp Gln Arg Pro Tyr Cys Trp His Tyr Ala
130 135 140

Pro Arg Pro Cys Gly Ile Val Pro Ala Ser Gln Val Cys Gly Pro Val
145 150 155 160

Tyr Cys Phe Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Phe
165 170 175

Gly Val Pro Thr Tyr Asn Trp Gly Ala Asn Asp Ser Asp Val Leu Ile
180 185 190

Leu Asn Asn Thr Arg Pro Pro Arg Gly Asn Trp Phe Gly Cys Thr Trp
195 200 205

Met Asn Gly Thr Gly Phe Thr Lys Thr Cys Gly Gly Pro Pro Cys Asn
210 215 220

Ile Gly Gly Ala Gly Asn Asn Thr Leu Thr Cys Pro Thr Asp Cys Phe
225 230 235 240

Arg Lys His Pro Glu Ala Thr Tyr Ala Arg Cys Gly Ser Gly Pro Trp
245 250 255

Leu Thr Pro Arg Cys Met Val His Tyr Pro Tyr Arg Leu Trp His Tyr
260 265 270

Pro Cys Thr Val Asn Phe Thr Ile Phe Lys Val Arg Met Tyr Val Gly
275 280 285

Gly Val Glu His Arg Phe Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu
290 295 300

Arg Cys Asp Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu
305 310 315 320

Leu Ser Thr Thr Glu Trp Gln Ile Leu Pro Cys Ser Phe Thr Thr Leu
325 330 335

Pro Ala Leu Ser Thr Gly Leu Ile His Leu His Gln Asn Ile Val Asp
340 345 350

Val Gln Tyr Leu Tyr Gly Val Gly Ser Ala Val Val Ser Leu Val Ile
355 360 355

Lys Trp Glu Tyr Val Leu Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg
370 375 380

Ile Cys Ala Cys Leu Trp Met Met Leu Leu Ile Ala Gln Ala Glu Ala
385 390 395 400

Ala Leu Glu Asn Leu Val Val Leu Asn Ala Ala Ala Val Ala Gly Ala
 405 410 415
 His Gly Thr Leu Ser Phe Leu Val Phe Phe Cys Ala Ala Trp Tyr Ile
 420 425 430
 Lys Gly Arg Leu Val Pro Gly Ala Ala Tyr Ala Phe Tyr Gly Val Trp
 435 440 445
 Pro Leu Leu Leu Leu Leu Ala Leu Pro Pro Arg Ala Tyr Ala
 450 455 460

(2) INFORMATION FOR SEQ ID NO: 47:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2082 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 1..2079

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
 (B) LOCATION: 1..2076

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

| | |
|---|-----|
| AAT TTG GGT AAG GTC ATC GAT ACC CTT ACA TGC GGC TTC GCC GAC CTC | 48 |
| Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu | |
| 1 5 10 15 | |
| GTG GGG TAC ATT CCG CTC GTC GGC GCC CCC CTA GGG GGC GCT GCC AGG | 96 |
| Val Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg | |
| 20 25 30 | |
| GCC CTG GCG CAT GGC GTC CGG GTT CTG GAG GAC GGC GTG AAC TAT GCA | 144 |
| Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala | |
| 35 40 45 | |
| ACA GGG AAT TTG CCC GGT TGC TCT TTC TCT ATC TTC CTC TTG GCT TTG | 192 |
| Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu | |
| 50 55 60 | |
| CTG TCC TGT CTG ACC GTT CCA GCT TCC GCT TAT GAA GTG CGC AAC GTG | 240 |
| Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr Glu Val Arg Asn Val | |
| 65 70 75 80 | |
| TCC GGG ATG TAC CAT GTC ACG AAC GAC TGC TCC AAC TCA AGC ATT GTG | 288 |
| Ser Gly Met Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val | |
| 85 90 95 | |
| TAT GAG GCA GCG GAC ATG ATC ATG CAC ACC CCC GGG TGC GTG CCC TGC | 336 |
| Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys | |
| 100 105 110 | |
| GTT CGG GAG AAC AAC TCT TCC CGC TGC TGG GTA GCG CTC ACC CCC ACG | 384 |
| Val Arg Glu Asn Asn Ser Ser Arg Cys Trp Val Ala Leu Thr Pro Thr | |

| 115 | 120 | 125 | |
|---|-----|-----|------|
| CTC GCA GCT AGG AAC GCC AGC GTC CCC ACC ACG ACA ATA CGA CGC CAC Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Ile Arg Arg His 130 135 140 | | | 432 |
| GTC GAT TTG CTC GTT GGG GCG GCT GCT TTC TGT TCC GCT ATG TAC GTG Val Asp Leu Leu Val Gly Ala Ala Ala Phe Cys Ser Ala Met Tyr Val 145 150 155 160 | | | 480 |
| GGG GAC CTC TGC GGA TCT GTC TTC CTC GTC TCC CAG CTG TTC ACC ATC Gly Asp Leu Cys Gly Ser Val Phe Leu Val Ser Gln Leu Phe Thr Ile 165 170 175 | | | 528 |
| TCG CCT CGC CGG CAT GAG ACG GTG CAG GAC TGC AAT TGC TCA ATC TAT Ser Pro Arg Arg His Glu Thr Val Gln Asp Cys Asn Cys Ser Ile Tyr 180 185 190 | | | 576 |
| CCC GGC CAC ATA ACG GGT CAC CGT ATG GCT TGG GAT ATG ATG ATG AAC Pro Gly His Ile Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn 195 200 205 | | | 624 |
| TGG TCG CCT ACA ACG GCC CTG GTG GTA TCG CAG CTG CTC CGG ATC CCA Trp Ser Pro Thr Thr Ala Leu Val Val Ser Gln Leu Leu Arg Ile Pro 210 215 220 | | | 672 |
| CAA GCT GTC GTG GAC ATG GTG GCG GGG GCC CAT TGG GGA GTC CTG GCG Gln Ala Val Val Asp Met Val Ala Gly Ala His Trp Gly Val Leu Ala 225 230 235 240 | | | 720 |
| GGC CTC GCC TAC TAT TCC ATG GTG GGG AAC TGG GCT AAG GTT TTG GTT Gly Leu Ala Tyr Tyr Ser Met Val Gly Asn Trp Ala Lys Val Leu Val 245 250 255 | | | 768 |
| GTG ATG CTA CTC TTT GCC GGC GTC GAC GGG CAT ACC CGC GTG TCA GGA Val Met Leu Leu Phe Ala Gly Val Asp Gly His Thr Arg Val Ser Gly 260 265 270 | | | 816 |
| GGG GCA GCA GCC TCC GAT ACC AGG GGC CTT GTG TCC CTC TTT AGC CCC Gly Ala Ala Ala Ser Asp Thr Arg Gly Leu Val Ser Leu Phe Ser Pro 275 280 285 | | | 864 |
| GGG TCG GCT CAG AAA ATC CAG CTC GTA AAC ACC AAC GGC AGT TGG CAC Gly Ser Ala Gln Lys Ile Gln Leu Val Asn Thr Asn Gly Ser Trp His 290 295 300 | | | 912 |
| ATC AAC AGG ACT GCC CTG AAC TGC AAC GAC TCC CTC CAA ACA GGG TTC Ile Asn Arg Thr Ala Leu Asn Cys Asn Asp Ser Leu Gln Thr Gly Phe 305 310 315 320 | | | 960 |
| TTT GCC GCA CTA TTC TAC AAA CAC AAA TTC AAC TCG TCT GGA TGC CCA Phe Ala Ala Leu Phe Tyr Lys His Lys Phe Asn Ser Ser Gly Cys Pro 325 330 335 | | | 1008 |
| GAG CGC TTG GCC AGC TGT CGC TCC ATC GAC AAG TTC GCT CAG GGG TGG Glu Arg Leu Ala Ser Cys Arg Ser Ile Asp Lys Phe Ala Gln Gly Trp 340 345 350 | | | 1056 |
| GGT CCC CTC ACT TAC ACT GAG CCT AAC AGC TCG GAC CAG AGG CCC TAC Gly Pro Leu Thr Tyr Thr Glu Pro Asn Ser Ser Asp Gln Arg Pro Tyr 355 360 365 | | | 1104 |
| TGC TGG CAC TAC GCG CCT CGA CCG TGT GGT ATT GTA CCC GCG TCT CAG Cys Trp His Tyr Ala Pro Arg Pro Cys Gly Ile Val Pro Ala Ser Gln 370 375 380 | | | 1152 |
| GTG TGC GGT CCA GTG TAT TGC TTC ACC CCG AGC CCT GTT GTG GTG GGG Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser Pro Val Val Val Gly 385 390 395 400 | | | 1200 |

| | |
|---|------|
| ACG ACC GAT CGG TTT GGT GTC CCC ACG TAT AAC TGG GGG GCG AAC GAC Thr Thr Asp Arg Phe Gly Val Pro Thr Tyr Asn Trp Gly Ala Asn Asp 405 410 415 | 1248 |
| TGG GAT GTG CTG ATT CTC AAC AAC ACG CGG CCG CCG CGA GGC AAC TGG Ser Asp Val Leu Ile Leu Asn Asn Thr Arg Pro Pro Arg Gly Asn Trp 420 425 430 | 1296 |
| TTC GGC TGT ACA TGG ATG AAT GGC ACT GGG TTC ACC AAG ACG TGT GGG Phe Gly Cys Thr Trp Met Asn Gly Thr Gly Phe Thr Lys Thr Cys Gly 435 440 445 | 1344 |
| GGC CCC CCG TGC AAC ATC GGG GGC GCC GGC AAC AAC ACC TTG ACC TGC Gly Pro Pro Cys Asn Ile Gly Gly Ala Gly Asn Asn Thr Leu Thr Cys 450 455 460 | 1392 |
| CCC ACT GAC TGT TTT CGG AAG CAC CCC GAG GCC ACC TAC GCC AGA TGC Pro Thr Asp Cys Phe Arg Lys His Pro Glu Ala Thr Tyr Ala Arg Cys 465 470 475 480 | 1440 |
| GGT TCT GGG CCC TGG CTG ACA CCT AGG TGT ATG GTT CAT TAC CCA TAT Gly Ser Gly Pro Trp Leu Thr Pro Arg Cys Met Val His Tyr Pro Tyr 485 490 495 | 1488 |
| AGG CTC TGG CAC TAC CCC TGC ACT GTC AAC TTC ACC ATC TTC AAG GTT Arg Leu Trp His Tyr Pro Cys Thr Val Asn Phe Thr Ile Phe Lys Val 500 505 510 | 1536 |
| AGG ATG TAC GTG GGG GGC GTG GAG CAC AGG TTC GAA GCC GCA TGC AAT Arg Met Tyr Val Gly Gly Val Glu His Arg Phe Glu Ala Ala Cys Asn 515 520 525 | 1584 |
| TGG ACT CGA GGA GAG CGT TGT GAC TTG GAG GAC AGG GAT AGA TCA GAG Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu Asp Arg Asp Arg Ser Glu 530 535 540 | 1632 |
| CTT AGC CCG CTG CTG CTG TCT ACA ACA GAG TGG CAG ATA CTG CCC TGT Leu Ser Pro Leu Leu Leu Ser Thr Thr Glu Trp Gln Ile Leu Pro Cys 545 550 555 | 1680 |
| TCC TTC ACC ACC CTG CCG GCC CTA TCC ACC GGC CTG ATC CAC CTC CAT Ser Phe Thr Thr Leu Pro Ala Leu Ser Thr Gly Leu Ile His Leu His 565 570 575 | 1728 |
| CAG AAC ATC GTG GAC GTG CAA TAC CTG TAC GGT GTA GGG TCG GCG GTT Gln Asn Ile Val Asp Val Gln Tyr Leu Tyr Gly Val Gly Ser Ala Val 580 585 590 | 1776 |
| GTC TCC CTT GTC ATC AAA TGG GAG TAT GTC CTG TTG CTC TTC CTT CTC Val Ser Leu Val Ile Lys Trp Glu Tyr Val Leu Leu Leu Phe Leu Leu 595 600 605 | 1824 |
| CTG GCA GAC GCG CGC ATC TGC GCC TGC TTA TGG ATG ATG CTG CTG ATA Leu Ala Asp Ala Arg Ile Cys Ala Cys Leu Trp Met Met Leu Leu Ile 610 615 620 | 1872 |
| GCT CAA GCT GAG GCC GCC TTA GAG AAC CTG GTG GTC CTC AAT GCG GCG Ala Gln Ala Glu Ala Ala Leu Glu Asn Leu Val Val Leu Asn Ala Ala 625 630 635 640 | 1920 |
| GCC GTG GCC GGG GCG CAT GGC ACT CTT TCC TTC CTT GTG TTC TTC TGT Ala Val Ala Gly Ala His Gly Thr Leu Ser Phe Leu Val Phe Phe Cys 645 650 655 | 1968 |
| GCT GCC TGG TAC ATC AAG GGC AGG CTG GTC CCT GGT GCG GCA TAC GCC Ala Ala Trp Tyr Ile Lys Gly Arg Leu Val Pro Gly Ala Ala Tyr Ala 660 665 670 | 2016 |
| TTC TAT GGC GTG TGG CCG CTG CTC CTG CTT CTG CTG GCC TTA CCA CCA | 2064 |

Phe Tyr Gly Val Trp Pro Leu Leu Leu Leu Leu Ala Leu Pro Pro
675 680 685

CGA GCT TAT GCC TAGTAA
Arg Ala Tyr Ala
690

2082

(2) INFORMATION FOR SEQ ID NO: 48:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 692 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu
1 5 10 15
Val Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg
20 25 30
Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala
35 40 45
Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu
50 55 60
Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr Glu Val Arg Asn Val
65 70 75 80
Ser Gly Met Tyr His Val Thr Asn Asp Cys Ser Asn Ser Ser Ile Val
85 90 95
Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys Val Pro Cys
100 105 110
Val Arg Glu Asn Asn Ser Ser Arg Cys Trp Val Ala Leu Thr Pro Thr
115 120 125
Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Thr Ile Arg Arg His
130 135 140
Val Asp Leu Leu Val Gly Ala Ala Ala Phe Cys Ser Ala Met Tyr Val
145 150 155 160
Gly Asp Leu Cys Gly Ser Val Phe Leu Val Ser Gln Leu Phe Thr Ile
165 170 175
Ser Pro Arg Arg His Glu Thr Val Gln Asp Cys Asn Cys Ser Ile Tyr
180 185 190
Pro Gly His Ile Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn
195 200 205
Trp Ser Pro Thr Thr Ala Leu Val Val Ser Gln Leu Leu Arg Ile Pro
210 215 220
Gln Ala Val Val Asp Met Val Ala Gly Ala His Trp Gly Val Leu Ala
225 230 235 240
Gly Leu Ala Tyr Tyr Ser Met Val Gly Asn Trp Ala Lys Val Leu Val
245 250 255
Val Met Leu Leu Phe Ala Gly Val Asp Gly His Thr Arg Val Ser Gly
260 265 270

- 130 -

Gly Ala Ala Ala Ser Asp Thr Arg Gly Leu Val Ser Leu Phe Ser Pro
275 280 285

Gly Ser Ala Gln Lys Ile Gln Leu Val Asn Thr Asn Gly Ser Trp His
290 295 300

Ile Asn Arg Thr Ala Leu Asn Cys Asn Asp Ser Leu Gln Thr Gly Phe
305 310 315 320

Phe Ala Ala Leu Phe Tyr Lys His Lys Phe Asn Ser Ser Gly Cys Pro
325 330 335

Glu Arg Leu Ala Ser Cys Arg Ser Ile Asp Lys Phe Ala Gln Gly Trp
340 345 350

Gly Pro Leu Thr Tyr Thr Glu Pro Asn Ser Ser Asp Gln Arg Pro Tyr
355 360 365

Cys Trp His Tyr Ala Pro Arg Pro Cys Gly Ile Val Pro Ala Ser Gln
370 375 380

Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser Pro Val Val Val Gly
385 390 395 400

Thr Thr Asp Arg Phe Gly Val Pro Thr Tyr Asn Trp Gly Ala Asn Asp
405 410 415

Ser Asp Val Leu Ile Leu Asn Asn Thr Arg Pro Pro Arg Gly Asn Trp
420 425 430

Phe Gly Cys Thr Trp Met Asn Gly Thr Gly Phe Thr Lys Thr Cys Gly
435 440 445

Gly Pro Pro Cys Asn Ile Gly Gly Ala Gly Asn Asn Thr Leu Thr Cys
450 455 460

Pro Thr Asp Cys Phe Arg Lys His Pro Glu Ala Thr Tyr Ala Arg Cys
465 470 475 480

Gly Ser Gly Pro Trp Leu Thr Pro Arg Cys Met Val His Tyr Pro Tyr
485 490 495

Arg Leu Trp His Tyr Pro Cys Thr Val Asn Phe Thr Ile Phe Lys Val
500 505 510

Arg Met Tyr Val Gly Gly Val Glu His Arg Phe Glu Ala Ala Cys Asn
515 520 525

Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu Asp Arg Asp Arg Ser Glu
530 535 540

Leu Ser Pro Leu Leu Leu Ser Thr Thr Glu Trp Gln Ile Leu Pro Cys
545 550 555 560

Ser Phe Thr Thr Leu Pro Ala Leu Ser Thr Gly Leu Ile His Leu His
565 570 575

Gln Asn Ile Val Asp Val Gln Tyr Leu Tyr Gly Val Gly Ser Ala Val
580 585 590

Val Ser Leu Val Ile Lys Trp Glu Tyr Val Leu Leu Phe Leu Leu
595 600 605

Leu Ala Asp Ala Arg Ile Cys Ala Cys Leu Trp Met Met Leu Leu Ile
610 615 620

Ala Gln Ala Glu Ala Ala Leu Glu Asn Leu Val Val Leu Asn Ala Ala
625 630 635 640

Ala Val Ala Gly Ala His Gly Thr Leu Ser Phe Leu Val Phe Phe Cys
645 650 655

Ala Ala Trp Tyr Ile Lys Gly Arg Leu Val Pro Gly Ala Ala Tyr Ala
660 665 670

Phe Tyr Gly Val Trp Pro Leu Leu Leu Leu Leu Ala Leu Pro Pro
675 680 685

Arg Ala Tyr Ala
690

(2) INFORMATION FOR SEQ ID NO: 49:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2433 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

- (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2430

- (ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 1..2427

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

| | |
|---|-----|
| ATG AGC ACG AAT CCT AAA CCT CAA AGA AAA ACC AAA CGT AAC ACC AAC | 48 |
| Met Ser Thr Asn Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn | |
| 1 5 10 15 | |
| CGC CGC CCA CAG GAC GTC AAG TTC CCG GGC GGT GGT CAG ATC GTT GGT | 96 |
| Arg Arg Pro Gln Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly | |
| 20 25 30 | |
| GGA GTT TAC CTG TTG CCG CGC AGG GGC CCC AGG TTG GGT GTG CGC GCG | 144 |
| Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala | |
| 35 40 45 | |
| ACT AGG AAG ACT TCC GAG CGG TCG CAA CCT CGT GGG AGG CGA CAA CCT | 192 |
| Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro | |
| 50 55 60 | |
| ATC CCC AAG GCT CGC CGA CCC GAG GGT AGG GCC TGG GCT CAG CCC GGG | 240 |
| Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Ala Trp Ala Gln Pro Gly | |
| 65 70 75 80 | |
| TAC CCT TGG CCC CTC TAT GGC AAT GAG GGC ATG GGG TGG GCA GGA TGG | 288 |
| Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Met Gly Trp Ala Gly Trp | |
| 85 90 95 | |
| CTC CTG TCA CCC CGC GGC TCT CGG CCT AGT TGG GGC CCT ACA GAC CCC | 336 |
| Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro | |
| 100 105 110 | |
| CGG CGT AGG TCG CGT AAT TTG GGT AAG GTC ATC GAT ACC CTT ACA TGC | 384 |
| Arg Arg Arg Ser Arg Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys | |
| 115 120 125 | |

| | | | | | | | | | | | | | | | | |
|-------------------|------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| GGC Gly 130 | TTC Phe | GCC Ala | GAC Asp | CTC Leu | GTG Val | GGG Gly 135 | TAC Tyr | ATT Ile | CCG Pro | CTC Leu | GTC Val 140 | GGC Gly | GCC Ala | CCC Pro | CTA Leu | 432 |
| GGG Gly 145 | GGC Gly | GCT Ala | GCC Ala | AGG Arg | GCC Ala 150 | CTG Leu | GCG Ala | CAT His | GGC Gly | GTC Val 155 | CGG Arg | GTT Val | CTG Leu | GAG Glu | GAC Asp 160 | 480 |
| GGC Gly | GTG Val | AAC Asn | TAT Tyr | GCA Ala 165 | ACA Thr | GGG Gly | AAT Asn | TTG Leu | CCC Pro 170 | GGT Gly | TGC Cys | TCT Ser | TTC Phe | TCT Ser 175 | ATC Ile | 528 |
| TTC Phe | CTC Leu | TTG Leu | GCT Ala 180 | TTG Leu | CTG Leu | TCC Ser | TGT Cys | CTG Leu 185 | ACC Thr | GTT Val | CCA Pro | GCT Ala | TCC Ser 190 | GCT Ala | TAT Tyr | 576 |
| GAA Glu | GTG Val | CGC Arg 195 | AAC Asn | GTG Val | TCC Ser | GGG Gly | ATG Met 200 | TAC Tyr | CAT His | GTC Val | ACG Thr 205 | AAC Asn | GAC Asp | TGC Cys | TCC Ser | 624 |
| AAC Asn 210 | TCA Ser | AGC Ser | ATT Ile | GTG Val | TAT Tyr | GAG Glu 215 | GCA Ala | GCG Ala | GAC Asp | ATG Met 220 | ATC Ile | ATG Met | CAC His | ACC Thr | CCC Pro | 672 |
| GGG Gly 225 | TGC Cys | GTG Val | CCC Pro | TGC Cys | GTT Val 230 | CGG Arg | GAG Glu | AAC Asn | AAC Asn | TCT Ser 235 | TCC Ser | CGC Arg | TGC Cys | TGG Trp 240 | GTA Val | 720 |
| GCG Ala | CTC Leu | ACC Thr | CCC Pro | ACG Thr 245 | CTC Leu | GCA Ala | GCT Ala | AGG Arg | AAC Asn 250 | GCC Ala | AGC Ser | GTC Val | CCC Pro | ACC Thr 255 | ACG Thr | 758 |
| ACA Thr | ATA Ile | CGA Arg | CGC Arg 260 | CAC His | GTC Val | GAT Asp | TTG Leu | CTC Leu 265 | GTT Val | GGG Gly | GCG Ala | GCT Ala | GCT Ala 270 | TTC Phe | TGT Cys | 816 |
| TCC Ser | GCT Ala | ATG Met 275 | TAC Tyr | GTG Val | GGG Gly | GAC Asp | CTC Leu 280 | TGC Cys | GGA Gly | TCT Ser | GTC Val | TTC Phe 285 | CTC Leu | GTC Val | TCC Ser | 864 |
| CAG Gln 290 | CTG Leu | TTC Phe | ACC Thr | ATC Ile | TCG Ser | CCT Pro 295 | CGC Arg | CGG Arg | CAT His | GAG Glu | ACG Thr 300 | GTG Val | CAG Gln | GAC Asp | TGC Cys | 912 |
| AAT Asn 305 | TGC Cys | TCA Ser | ATC Ile | TAT Tyr | CCC Pro 310 | GGC Gly | CAC His | ATA Ile | ACG Thr 315 | GGT Gly | CAC His | CGT Arg | ATG Met | GCT Ala 320 | TGG Trp | 960 |
| GAT Asp | ATG Met | ATG Met | ATG Met | AAC Asn 325 | TGG Trp | TCG Ser | CCT Pro | ACA Thr | ACG Thr 330 | GCC Ala | CTG Leu | GTG Val | GTA Val | TCG Ser 335 | CAG Gln | 1008 |
| CTG Leu | CTC Leu | CGG Arg | ATC Ile 340 | CCA Pro | CAA Gln | GCT Ala | GTC Val 345 | GTG Val | GAC Asp | ATG Met | GTG Val | GCG Ala | GGG Gly 350 | GCC Ala | CAT His | 1056 |
| TGG Trp | GGA Gly | GTC Val 355 | CTG Leu | GCG Ala | GGC Gly | CTC Leu | GCC Ala 360 | TAC Tyr | TAT Tyr | TCC Ser | ATG Met | GTG Val 365 | GGG Gly | AAC Asn | TGG Trp | 1104 |
| GCT Ala 370 | AAG Val | GTT Leu | TTG Val | GTT Val | GTG Val 375 | ATG Leu | CTA Leu | CTC Leu | TTT Phe | GCC Ala 380 | GGC Gly | GTC Val | GAC Asp | GGG Gly | CAT His | 1152 |
| ACC Thr 385 | CGC Arg | GTG Val | TCA Ser | GGA Gly | GGG Gly 390 | GCA Ala | GCA Ala | GCC Ala | TCC Ser | GAT Asp 395 | ACC Thr | AGG Arg | GGC Gly | CTT Leu | GTG Val 400 | 1200 |
| TCC Leu | CTC Leu | TTT Phe | AGC Thr | CCC Pro | GGG Gly | TCG Ser | GCT Pro | CAG Thr | AAA Ala | ATC Ala | CAG Thr | CTC Leu | GTA Val | AAC Asn | ACC Ser | 1248 |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| Ser | Leu | Phe | Ser | Pro | Gly | Ser | Ala | Gln | Lys | Ile | Gln | Leu | Val | Asn | Thr | |
| | | | | 405 | | | | | 410 | | | | | 415 | | |
| AAC | GGC | AGT | TGG | CAC | ATC | AAC | AGG | ACT | GCC | CTG | AAC | TGC | AAC | GAC | TCC | 1296 |
| Asn | Gly | Ser | Trp | His | Ile | Asn | Arg | Thr | Ala | Leu | Asn | Cys | Asn | Asp | Ser | |
| | | | 420 | | | | | 425 | | | | | 430 | | | |
| CTC | CAA | ACA | GGG | TTC | TTT | GCC | GCA | CTA | TTC | TAC | AAA | CAC | AAA | TTC | AAC | 1344 |
| Leu | Gln | Thr | Gly | Phe | Phe | Ala | Ala | Leu | Phe | Tyr | Lys | His | Lys | Phe | Asn | |
| | | | 435 | | | | 440 | | | | | 445 | | | | |
| TCG | TCT | GGA | TGC | CCA | GAG | CGC | TTC | GCC | AGC | TGT | CGC | TCC | ATC | GAC | AAG | 1392 |
| Ser | Ser | Gly | Cys | Pro | Glu | Arg | Leu | Ala | Ser | Cys | Arg | Ser | Ile | Asp | Lys | |
| | | | 450 | | | 455 | | | | | 460 | | | | | |
| TTC | GCT | CAG | GGG | TGG | GTT | CCC | CTC | ACT | TAC | ACT | GAG | CCT | AAC | AGC | TCG | 1440 |
| Phe | Ala | Gln | Gly | Trp | Gly | Pro | Leu | Thr | Tyr | Thr | Glu | Pro | Asn | Ser | Ser | |
| | | | 465 | | 470 | | | | | 475 | | | | | 480 | |
| GAC | CAG | AGG | CCC | TAC | TGC | TGG | CAC | TAC | GCG | CCT | CGA | CCG | TGT | GGT | ATT | 1488 |
| Asp | Gln | Arg | Pro | Tyr | Cys | Trp | His | Tyr | Ala | Pro | Arg | Pro | Cys | Gly | Ile | |
| | | | | 485 | | | | | 490 | | | | | 495 | | |
| GTA | CCC | GCG | TCT | CAG | GTG | TGC | GGT | CCA | GTG | TAT | TGC | TTC | ACC | CCG | AGC | 1536 |
| Val | Pro | Ala | Ser | Gln | Val | Cys | Gly | Pro | Val | Tyr | Cys | Phe | Thr | Pro | Ser | |
| | | | 500 | | | | | 505 | | | | | 510 | | | |
| CCT | GTT | GTG | GTG | GGG | ACG | ACC | GAT | CGG | TTT | GGT | GTC | CCC | ACG | TAT | AAC | 1584 |
| Pro | Val | Val | Val | Gly | Thr | Thr | Asp | Arg | Phe | Gly | Val | Pro | Thr | Tyr | Asn | |
| | | | 515 | | | | 520 | | | | | 525 | | | | |
| TGG | GGG | GCG | AAC | GAC | TCG | GAT | GTG | CTG | ATT | CTC | AAC | AAC | ACG | CGG | CCG | 1632 |
| Trp | Gly | Ala | Asn | Asp | Ser | Asp | Val | Leu | Ile | Leu | Asn | Asn | Thr | Arg | Pro | |
| | | | 530 | | | 535 | | | | | 540 | | | | | |
| CCG | CGA | GGC | AAC | TGG | TTC | GGC | TGT | ACA | TGG | ATG | AAT | GGC | ACT | GGG | TTC | 1680 |
| Pro | Arg | Gly | Asn | Trp | Phe | Gly | Cys | Thr | Trp | Met | Asn | Gly | Thr | Gly | Phe | |
| | | | | | 550 | | | | | 555 | | | | | 560 | |
| ACC | AAG | ACG | TGT | GGG | GGC | CCC | CCG | TGC | AAC | ATC | GGG | GGG | GCC | GGC | AAC | 1728 |
| Thr | Lys | Thr | Cys | Gly | Gly | Pro | Pro | Cys | Asn | Ile | Gly | Gly | Ala | Gly | Asn | |
| | | | | 565 | | | | | 570 | | | | | 575 | | |
| AAC | ACC | TTG | ACC | TGC | CCC | ACT | GAC | TGT | TTT | CGG | AAG | CAC | CCC | GAG | GCC | 1776 |
| Asn | Thr | Leu | Thr | Cys | Pro | Thr | Asp | Cys | Phe | Arg | Lys | His | Pro | Glu | Ala | |
| | | | | 580 | | | | 585 | | | | | 590 | | | |
| ACC | TAC | GCC | AGA | TGC | GGT | TCT | GGG | CCC | TGG | CTG | ACA | CCT | AGG | TGT | ATG | 1824 |
| Thr | Tyr | Ala | Arg | Cys | Gly | Ser | Gly | Pro | Trp | Leu | Thr | Pro | Arg | Cys | Met | |
| | | | 595 | | | | 600 | | | | | 605 | | | | |
| GTT | CAT | TAC | CCA | TAT | AGG | CTC | TGG | CAC | TAC | CCC | TGC | ACT | GTC | AAC | TTC | 1872 |
| Val | His | Tyr | Pro | Tyr | Arg | Leu | Trp | His | Tyr | Pro | Cys | Thr | Val | Asn | Phe | |
| | | | 610 | | | 615 | | | | | 620 | | | | | |
| ACC | ATC | TTC | AAG | GTT | AGG | ATG | TAC | GTG | GGG | GGC | GTG | GAG | CAC | AGG | TTC | 1920 |
| Thr | Ile | Phe | Lys | Val | Arg | Met | Tyr | Val | Gly | Gly | Val | Glu | His | Arg | Phe | |
| | | | | | 630 | | | | 635 | | | | | | 640 | |
| GAA | GCC | GCA | TGC | AAT | TGG | ACT | CGA | GGA | GAG | CGT | TGT | GAC | TTG | GAG | GAC | 1968 |
| Glu | Ala | Ala | Cys | Asn | Trp | Thr | Arg | Gly | Glu | Arg | Cys | Asp | Leu | Glu | Asp | |
| | | | | 645 | | | | 650 | | | | | 655 | | | |
| AGG | GAT | AGA | TCA | GAG | CTT | AGC | CCG | CTG | CTG | CTG | TCT | ACA | ACA | GAG | TGG | 2016 |
| Arg | Asp | Arg | Ser | Glu | Leu | Ser | Pro | Leu | Leu | Leu | Ser | Thr | Thr | Glu | Trp | |
| | | | 660 | | | | 665 | | | | | | 670 | | | |
| CAG | ATA | CTG | CCC | TGT | TCC | TTC | ACC | ACC | CTG | CCG | GCC | CTA | TCC | ACC | GGC | 2064 |
| Gln | Ile | Leu | Pro | Cys | Ser | Phe | Thr | Thr | Leu | Pro | Ala | Leu | Ser | Thr | Gly | |

| 675 | | | | | | | | | | 680 | | | | | | | | | | 685 | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------|-----|-----|-----|-----|-----|-----|------|--|--|--|-----|--|--|--|--|--|--|--|--|--|--|
| CTG | ATC | CAC | CTC | CAT | CAG | AAC | ATC | GTG | GAC | GTG | CAA | TAC | CTG | TAC | GGT | 2112 | | | | | | | | | | | | | | |
| Leu | Ile | His | Leu | His | Gln | Asn | Ile | Val | Asp | Val | Gln | Tyr | Leu | Tyr | Gly | | | | | | | | | | | | | | | |
| 690 | | | | | | 695 | | | | | 700 | | | | | | | | | | | | | | | | | | | |
| GTA | GGG | TCG | GCG | GTT | GTC | TCC | CTT | GTG | ATC | AAA | TGG | GAG | TAT | GTC | CTG | 2160 | | | | | | | | | | | | | | |
| Val | Gly | Ser | Ala | Val | Val | Ser | Leu | Val | Ile | Lys | Trp | Glu | Tyr | Val | Leu | | | | | | | | | | | | | | | |
| 705 | | | | | 710 | | | | | 715 | | | | | 720 | | | | | | | | | | | | | | | |
| TTG | CTC | TTC | CTT | CTC | CTG | GCA | GAC | GCG | CGC | ATC | TGC | GCC | TGC | TTA | TGG | 2208 | | | | | | | | | | | | | | |
| Leu | Leu | Phe | Leu | Leu | Leu | Ala | Asp | Ala | Arg | Ile | Cys | Ala | Cys | Leu | Trp | | | | | | | | | | | | | | | |
| | | | | 725 | | | | | 730 | | | | | 735 | | | | | | | | | | | | | | | | |
| ATG | ATG | CTG | CTG | ATA | GCT | CAA | GCT | GAG | GCC | GCC | TTA | GAG | AAC | CTG | GTG | 2256 | | | | | | | | | | | | | | |
| Met | Met | Leu | | Ile | Ala | Gln | Ala | Glu | Ala | Ala | Leu | Glu | Asn | Leu | Val | | | | | | | | | | | | | | | |
| | | | 740 | | | | | 745 | | | | | 750 | | | | | | | | | | | | | | | | | |
| GTC | CTC | AAT | GCG | GCG | GCC | GTG | GCC | GGG | GCG | CAT | GGC | ACT | CTT | TCC | TTC | 2304 | | | | | | | | | | | | | | |
| Val | Leu | Asn | Ala | Ala | Ala | Val | Ala | Gly | Ala | His | Gly | Thr | Leu | Ser | Phe | | | | | | | | | | | | | | | |
| | | 755 | | | | | 760 | | | | | 765 | | | | | | | | | | | | | | | | | | |
| CTT | GTG | TTC | TTC | TGT | GCT | GCC | TGG | TAC | ATC | AAG | GGC | AGG | CTG | GTC | CCT | 2352 | | | | | | | | | | | | | | |
| Leu | Val | Phe | Phe | Cys | Ala | Ala | Trp | Tyr | Ile | Lys | Gly | Arg | Leu | Val | Pro | | | | | | | | | | | | | | | |
| | 770 | | | | 775 | | | | | | 780 | | | | | | | | | | | | | | | | | | | |
| GGT | GCG | GCA | TAC | GCC | TTC | TAT | GGC | GTG | TGG | CCG | CTG | CTC | CTG | CTT | CTG | 2400 | | | | | | | | | | | | | | |
| Gly | Ala | Ala | Tyr | Ala | Phe | Tyr | Gly | Val | Trp | Pro | Leu | Leu | Leu | Leu | Leu | | | | | | | | | | | | | | | |
| 785 | | | | 790 | | | | | 795 | | | | | | 800 | | | | | | | | | | | | | | | |
| CTG | GCC | TTA | CCA | CCA | CGA | GCT | TAT | GCC | TAGTAA | | | | | | | 2433 | | | | | | | | | | | | | | |
| Leu | Ala | Leu | Pro | Pro | Arg | Ala | Tyr | Ala | | | | | | | | | | | | | | | | | | | | | | |
| | | | | 805 | | | | 810 | | | | | | | | | | | | | | | | | | | | | | |

(2) INFORMATION FOR SEQ ID NO: 50:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 809 amino acids
 (B) TYPE: amino acid.
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ser | Thr | Asn | Pro | Lys | Pro | Gln | Arg | Lys | Thr | Lys | Arg | Asn | Thr | Asn |
| 1 | | | | 5 | | | | | 10 | | | | 15 | | |
| Arg | Arg | Pro | Gln | Asp | Val | Lys | Phe | Pro | Gly | Gly | Gly | Gln | Ile | Val | Gly |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| Gly | Val | Tyr | Leu | Leu | Pro | Arg | Arg | Gly | Pro | Arg | Leu | Gly | Val | Arg | Ala |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Thr | Arg | Lys | Thr | Ser | Glu | Arg | Ser | Gln | Pro | Arg | Gly | Arg | Arg | Gln | Pro |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Ile | Pro | Lys | Ala | Arg | Arg | Pro | Glu | Gly | Arg | Ala | Trp | Ala | Gln | Pro | Gly |
| | 65 | | | | 70 | | | | 75 | | | | | 80 | |
| Tyr | Pro | Trp | Pro | Leu | Tyr | Gly | Asn | Glu | Gly | Met | Gly | Trp | Ala | Gly | Trp |
| | | | 85 | | | | | 90 | | | | | 95 | | |
| Leu | Leu | Ser | Pro | Arg | Gly | Ser | Arg | Pro | Ser | Trp | Gly | Pro | Thr | Asp | Pro |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Arg | Arg | Arg | Ser | Arg | Asn | Leu | Gly | Lys | Val | Ile | Asp | Thr | Leu | Thr | Cys |
| | | | 115 | | | | 120 | | | | 125 | | | | |

Gly Phe Ala Asp Leu Val Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu
130 135 140

Gly Gly Ala Ala Arg Ala Leu Ala His Gly Val Arg Val Leu Glu Asp
145 150 155 160

Gly Val Asn Tyr Ala Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile
165 170 175

Phe Leu Leu Ala Leu Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr
180 185 190

Glu Val Arg Asn Val Ser Gly Met Tyr His Val Thr Asn Asp Cys Ser
195 200 205

Asn Ser Ser Ile Val Tyr Glu Ala Ala Asp Met Ile Met His Thr Pro
210 215 220

Gly Cys Val Pro Cys Val Arg Glu Asn Asn Ser Ser Arg Cys Trp Val
225 230 235 240

Ala Leu Thr Pro Thr Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr
245 250 255

Thr Ile Arg Arg His Val Asp Leu Leu Val Gly Ala Ala Ala Phe Cys
260 265 270

Ser Ala Met Tyr Val Gly Asp Leu Cys Gly Ser Val Phe Leu Val Ser
275 280 285

Gln Leu Phe Thr Ile Ser Pro Arg Arg His Glu Thr Val Gln Asp Cys
290 295 300

Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg Met Ala Trp
305 310 315 320

Asp Met Met Met Asn Trp Ser Pro Thr Thr Ala Leu Val Val Ser Gln
325 330 335

Leu Leu Arg Ile Pro Gln Ala Val Val Asp Met Val Ala Gly Ala His
340 345 350

Trp Gly Val Leu Ala Gly Leu Ala Tyr Tyr Ser Met Val Gly Asn Trp
355 360 365

Ala Lys Val Leu Val Val Met Leu Leu Phe Ala Gly Val Asp Gly His
370 375 380

Thr Arg Val Ser Gly Gly Ala Ala Ala Ser Asp Thr Arg Gly Leu Val
385 390 395 400

Ser Leu Phe Ser Pro Gly Ser Ala Gln Lys Ile Gln Leu Val Asn Thr
405 410 415

Asn Gly Ser Trp His Ile Asn Arg Thr Ala Leu Asn Cys Asn Asp Ser
420 425 430

Leu Gln Thr Gly Phe Phe Ala Ala Leu Phe Tyr Lys His Lys Phe Asn
435 440 445

Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys Arg Ser Ile Asp Lys
450 455 460

Phe Ala Gln Gly Trp Gly Pro Leu Thr Tyr Thr Glu Pro Asn Ser Ser
465 470 475 480

Asp Gln Arg Pro Tyr Cys Trp His Tyr Ala Pro Arg Pro Cys Gly Ile
485 490 495

Val Pro Ala Ser Gln Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser
500 505 510

Pro Val Val Val Gly Thr Thr Asp Arg Phe Gly Val Pro Thr Tyr Asn
515 520 525

Trp Gly Ala Asn Asp Ser Asp Val Leu Ile Leu Asn Asn Thr Arg Pro
530 535 540

Pro Arg Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Gly Thr Gly Phe
545 550 555 560

Thr Lys Thr Cys Gly Gly Pro Pro Cys Asn Ile Gly Gly Ala Gly Asn
565 570 575

Asn Thr Leu Thr Cys Pro Thr Asp Cys Phe Arg Lys His Pro Glu Ala
580 585 590

Thr Tyr Ala Arg Cys Gly Ser Gly Pro Trp Leu Thr Pro Arg Cys Met
595 600 605

Val His Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Val Asn Phe
610 615 620

Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly Val Glu His Arg Phe
625 630 635 640

Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu Asp
645 650 655

Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu Ser Thr Thr Glu Trp
660 665 670

Gln Ile Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala Leu Ser Thr Gly
675 680 685

Leu Ile His Leu His Gln Asn Ile Val Asp Val Gln Tyr Leu Tyr Gly
690 695 700

Val Gly Ser Ala Val Val Ser Leu Val Ile Lys Trp Glu Tyr Val Leu
705 710 715 720

Leu Leu Phe Leu Leu Ala Asp Ala Arg Ile Cys Ala Cys Leu Trp
725 730 735

Met Met Leu Leu Ile Ala Gln Ala Glu Ala Ala Leu Glu Asn Leu Val
740 745 750

Val Leu Asn Ala Ala Ala Val Ala Gly Ala His Gly Thr Leu Ser Phe
755 760 765

Leu Val Phe Phe Cys Ala Ala Trp Tyr Ile Lys Gly Arg Leu Val Pro
770 775 780

Gly Ala Ala Tyr Ala Phe Tyr Gly Val Trp Pro Leu Leu Leu Leu Leu
785 790 795 800

Leu Ala Leu Pro Pro Arg Ala Tyr Ala
805

(2) INFORMATION FOR SEQ ID NO: 51:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 17 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1..17

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

Ser Asn Ser Ser Glu Ala Ala Asp Met Ile Met His Thr Pro Gly Cys
 1 5 10 15
 Val

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1..22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

Gly Gly Ile Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn Trp
 1 5 10 15
 Ser Pro Thr Thr Ala Leu
 20

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 1..37

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

Tyr Glu Val Arg Asn Val Ser Gly Ile Tyr His Val Thr Asn Asp Cys
 1 5 10 15
 Ser Asn Ser Ser Ile Val Tyr Glu Ala Ala Asp Met Ile Met His Thr
 20 25 30
 Pro Gly Cys Gly Lys
 35

(2) INFORMATION FOR SEQ ID NO: 54:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:
 - (A) NAME/KEY: Modified-site
 - (B) LOCATION: 1..25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

Gly Gly Thr Pro Thr Val Ala Thr Arg Asp Gly Lys Leu Pro Ala Thr
 1 5 10 15
 Gln Leu Arg Arg His Ile Asp Leu Leu
 20 25

(2) INFORMATION FOR SEQ ID NO: 55:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:
 - (A) NAME/KEY: Modified-site
 - (B) LOCATION: 1..25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

Gly Gly Thr Pro Thr Leu Ala Ala Arg Asp Ala Ser Val Pro Thr Thr
 1 5 10 15
 Thr Ile Arg Arg His Val Asp Leu Leu
 20 25

(2) INFORMATION FOR SEQ ID NO: 56:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

Leu Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr Gln Val Arg Asn
 1 5 10 15
 Ser Thr Gly Leu
 20

(2) INFORMATION FOR SEQ ID NO: 57:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

Gln Val Arg Asn Ser Thr Gly Leu Tyr His Val Thr Asn Asp Cys Pro
1 5 10 15
Asn Ser Ser Ile
 20

(2) INFORMATION FOR SEQ ID NO: 58:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

Asn Asp Cys Pro Asn Ser Ser Ile Val Tyr Glu Ala His Asp Ala Ile
1 5 10 15
Leu His Thr Pro
 20

(2) INFORMATION FOR SEQ ID NO: 59:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

Ser Asn Ser Ser Ile Val Tyr Glu Ala Ala Asp Met Ile Met His Thr
1 5 10 15
Pro Gly Cys Val
 20

(2) INFORMATION FOR SEQ ID NO: 60:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

His Asp Ala Ile Leu His Thr Pro Gly Val Pro Cys Val Arg Glu Gly
 1 5 10 15
 Asn Val Ser

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

Cys Val Arg Glu Gly Asn Val Ser Arg Cys Trp Val Ala Met Thr Pro
 1 5 10 15
 Thr Val Ala Thr
 20

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

Ala Met Thr Pro Thr Val Ala Thr Arg Asp Gly Lys Leu Pro Ala Thr
 1 5 10 15
 Gln Leu Arg Arg
 20

(2) INFORMATION FOR SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

Leu Pro Ala Thr Gln Leu Arg Arg His Ile Asp Leu Leu Val Gly Ser
 1 5 10 15
 Ala Thr Leu Cys
 20

(2) INFORMATION FOR SEQ ID NO: 64:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

Leu Val Gly Ser Ala Thr Leu Cys Ser Ala Leu Tyr Val Gly Asp Leu
1 5 10 15
Cys Gly Ser Val
20

(2) INFORMATION FOR SEQ ID NO: 65:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

Gln Leu Phe Thr Phe Ser Pro Arg Arg His Trp Thr Thr Gln Gly Cys
1 5 10 15
Asn Cys Ser Ile
20

(2) INFORMATION FOR SEQ ID NO: 66:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

Thr Gln Gly Cys Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His
1 5 10 15
Arg Met Ala Trp
20

(2) INFORMATION FOR SEQ ID NO: 67:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

Ile Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn Trp Ser Pro
1 5 10 15
Thr Ala Ala Leu
20

(2) INFORMATION FOR SEQ ID NO: 68:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

Asn Trp Ser Pro Thr Ala Ala Leu Val Met Ala Gln Leu Leu Arg Ile
1 5 10 15
Pro Gln Ala Ile
20

(2) INFORMATION FOR SEQ ID NO: 69:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

Leu Leu Arg Ile Pro Gln Ala Ile Leu Asp Met Ile Ala Gly Ala His
1 5 10 15
Trp Gly Val Leu
20

(2) INFORMATION FOR SEQ ID NO: 70:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

Ala Gly Ala His Trp Gly Val Leu Ala Gly Ile Ala Tyr Phe Ser Met
1 5 10 15
Val Gly Asn Met
20

(2) INFORMATION FOR SEQ ID NO: 71:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

Val Val Leu Leu Leu Phe Ala Gly Val Asp Ala Glu Thr Ile Val Ser
1 5 10 15
Gly Gly Gln Ala
 20

(2) INFORMATION FOR SEQ ID NO: 72:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

Ser Gly Leu Val Ser Leu Phe Thr Pro Gly Ala Lys Gln Asn Ile Gln
1 5 10 15
Leu Ile Asn Thr
 20

(2) INFORMATION FOR SEQ ID NO: 73:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

Gln Asn Ile Gln Leu Ile Asn Thr Asn Gly Gln Trp His Ile Asn Ser
1 5 10 15
Thr Ala Leu Asn
 20

(2) INFORMATION FOR SEQ ID NO: 74:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

Leu Asn Cys Asn Glu Ser Leu Asn Thr Gly Trp Trp Leu Ala Gly Leu
1 5 10 15
Ile Tyr Gln His Lys
20

(2) INFORMATION FOR SEQ ID NO: 75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

Ala Gly Leu Ile Tyr Gln His Lys Phe Asn Ser Ser Gly Cys Pro Glu
1 5 10 15
Arg Leu Ala Ser
20

(2) INFORMATION FOR SEQ ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:

Gly Cys Pro Glu Arg Leu Ala Ser Cys Arg Pro Leu Thr Asp Phe Asp
1 5 10 15
Gln Gly Trp Gly
20

(2) INFORMATION FOR SEQ ID NO: 77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:

Thr Asp Phe Asp Gln Gly Trp Gly Pro Ile Ser Tyr Ala Asn Gly Ser
1 5 10 15
Gly Pro Asp Gln
20

(2) INFORMATION FOR SEQ ID NO: 78:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:

Ala Asn Gly Ser Gly Pro Asp Gln Arg Pro Tyr Cys Trp His Tyr Pro
1 5 10 15
Pro Lys Pro Cys
 20

(2) INFORMATION FOR SEQ ID NO: 79:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:

Trp His Tyr Pro Pro Lys Pro Cys Gly Ile Val Pro Ala Lys Ser Val
1 5 10 15
Cys Gly Pro Val
 20

(2) INFORMATION FOR SEQ ID NO: 80:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:

Ala Lys Ser Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser Pro Val
1 5 10 15
Val Val Gly Thr
 20

(2) INFORMATION FOR SEQ ID NO: 81:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:

Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Ser Gly Ala Pro Thr
 1 5 10 15
 Tyr Ser Trp Gly
 20

(2) INFORMATION FOR SEQ ID NO: 82:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 82:

Gly Ala Pro Thr Tyr Ser Trp Gly Glu Asn Asp Thr Asp Val Phe Val
 1 5 10 15
 Leu Asn Asn Thr
 20

(2) INFORMATION FOR SEQ ID NO: 83:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 83:

Gly Asn Trp Phe Gly Cys Thr Trp Met Asn Ser Thr Gly Phe Thr Lys
 1 5 10 15
 Val Cys Gly Ala
 20

(2) INFORMATION FOR SEQ ID NO: 84:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 84:

Gly Phe Thr Lys Val Cys Gly Ala Pro Pro Val Cys Ile Gly Gly Ala
 1 5 10 15
 Gly Asn Asn Thr
 20

(2) INFORMATION FOR SEQ ID NO: 85:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 85:

Ile Gly Gly Ala Gly Asn Asn Thr Leu His Cys Pro Thr Asp Cys Arg
1 5 10 15
Lys His Pro

(2) INFORMATION FOR SEQ ID NO: 86:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 86:

Thr Asp Cys Phe Arg Lys His Pro Asp Ala Thr Tyr Ser Arg Cys Gly
1 5 10 15
Ser Gly Pro Trp
 20

(2) INFORMATION FOR SEQ ID NO: 87:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 87:

Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr Pro Arg Cys Leu Val Asp
1 5 10 15
Tyr Pro Tyr Arg
 20

(2) INFORMATION FOR SEQ ID NO: 88:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 88:

Cys Leu Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Ile
1 5 10 15
Asn Tyr Thr Ile
20

(2) INFORMATION FOR SEQ ID NO: 89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 89:

Pro Cys Thr Ile Asn Tyr Thr Ile Phe Lys Ile Arg Met Tyr Val Gly
1 5 10 15
Gly Val Glu His
20

(2) INFORMATION FOR SEQ ID NO: 90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 90:

Met Tyr Val Gly Gly Val Glu His Arg Leu Glu Ala Ala Cys Asn Trp
1 5 10 15
Thr Pro Gly Glu
20

(2) INFORMATION FOR SEQ ID NO: 91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 91:

Ala Cys Asn Trp Thr Pro Gly Glu Arg Cys Asp Leu Glu Asp Arg Asp
1 5 10 15
Arg Ser Glu Leu
20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 92:

Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu Thr Thr Thr
1 5 10 15
Gln Trp Gln Val
20

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 93:

Tyr Gln Val Arg Asn Ser Thr Gly Leu
1 5

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 29 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 94:

29

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 95:

CCTCCGGACG TGCACTAGCT CCCGTCGTG GTAGTGGTGG TAGTGATTAT CAATTAATTG

60

(2) INFORMATION FOR SEQ ID NO: 96:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 96:

GTTTAACCAC TGCATGATG

19

(2) INFORMATION FOR SEQ ID NO: 97:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 97:

GTCCCATCGA GTGCGGCTAC

20

(2) INFORMATION FOR SEQ ID NO: 98:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 98:

CGTGACATGG TACATTCCGG ACACTTGGCG CACTTCATAA GCGGA

45

(2) INFORMATION FOR SEQ ID NO: 99:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 42 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 99:

TGCCTCATAC ACAATGGAGC TCTGGGACGA GTCGTTCTGTG AC

42

(2) INFORMATION FOR SEQ ID NO: 100:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 42 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 100:

TACCCAGCAG CGGGAGCTCT GTTGCTCCCG AACGCAGGGC AC

42

(2) INFORMATION FOR SEQ ID NO: 101:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 42 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 101:

TGTCGTGGTG GGGACGGAGG CCTGCCTAGC TGCGAGCGTG GG

42

(2) INFORMATION FOR SEQ ID NO: 102:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 48 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 102:

CGTTATGTGG CCCGGGTAGA TTGAGCACTG GCAGTCCTGC ACCGTCTC

48

(2) INFORMATION FOR SEQ ID NO: 103:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 42 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 103:

CAGGGCCGTT CTAGGCCTCC ACTGCATCAT CATATCCCAA GC

42

(2) INFORMATION FOR SEQ ID NO: 104:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 104:

CCGGAATGTA CCATGTCACG AACGAC

26

(2) INFORMATION FOR SEQ ID NO: 105:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 105:

GCTCCATTGT GTATGAGGCA GCGG

24

(2) INFORMATION FOR SEQ ID NO: 106:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 106:

GAGCTCCCGC TGCTGGGTAG CGC

23

(2) INFORMATION FOR SEQ ID NO: 107:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 107:

CCTCCGTCCC CACCAGACA ATACG

25

(2) INFORMATION FOR SEQ ID NO: 108:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 108:

CTACCCGGGC CACATAACGG GTCACCG

27

(2) INFORMATION FOR SEQ ID NO: 109:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 109:

GGAGGCCTAC AACGGCCCTG GTGG

24

(2) INFORMATION FOR SEQ ID NO: 110:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 110:

TTCTATCGAT TAAATAGAAT TC

22

(2) INFORMATION FOR SEQ ID NO: 111:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 111:

GCCATACGCT CACAGCCGAT CCC

23

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. An isolated HCV single or specific oligomeric envelope protein selected from the group consisting of E1 and/or E2 and/or E1/E2, having a purity degree of at least 80%.
- 5 2. An isolated HCV single or specific oligomeric envelope protein selected from the group consisting of E1 and/or E2 and/or E1/E2, having a purity degree of at least 90%.
3. An isolated HCV single or specific oligomeric envelope protein selected from the group consisting of E1 and/or E2 and/or E1/E2, having a purity degree of at least 95%.
4. An isolated HCV envelope protein according to any one of claims 1 to 3, wherein
10 said isolated HCV envelope proteins are expressed from recombinant mammalian cells such as by using a vaccinia virus based system.
5. An isolated HCV envelope protein according to claim 4, wherein said isolated HCV envelope proteins are expressed from recombinant yeast cells.
6. An isolated HCV envelope protein according to any one of claims 1 to 5, for use
15 as a medicament.
7. An isolated HCV envelope protein according to any one of claims 1 to 5, for use as a vaccine for immunizing a mammal against HCV, comprising administering an effective amount of said composition, optionally accompanied by pharmaceutically acceptable adjuvants, to produce an immune response
- 20 8. An isolated HCV envelope protein according to claim 7 wherein said mammal is human.
9. A method for immunising a mammal against HCV, comprising the steps of administering to said mammal an effective amount of an isolated HCV envelope protein according to any one of claims 1 to 5, to produce an immune response.

10. A method according to claim 9, wherein said mammal is human.
11. A vaccine composition for immunizing a mammal against HCV, comprising an effective amount of an isolated HCV envelope protein according to any one of claims 1 to 5, optionally accompanied by pharmaceutically acceptable adjuvants.
- 5 12. A vaccine composition according to claim 11 wherein said mammal is human.
13. An isolated HCV envelope protein according to any one of claims 1 to 5, for *in vitro* detection of HCV antibodies present in a biological sample.
14. A method for *in vitro* diagnosis of HCV antibodies present in a biological sample, comprising at least the following steps:
- 10 (i) contacting said biological sample with an isolated HCV envelope protein according to any one of claims 1 to 5, under appropriate conditions which allow the formation of an immune complex,
- (ii) removing unbound components,
- (iii) incubating the immune complexes formed with heterologous antibodies,
- 15 with said heterologous antibodies being conjugated to a detectable label under appropriate conditions,
- (iv) detecting the presence of said immune complexes visually or mechanically.
15. A method according to claim 14 wherein said isolated HCV envelope protein is
- 20 in an immobilised form.
16. A kit for determining the presence of HCV antibodies present in a biological sample, comprising:

- at least one isolated HCV envelope protein according to any one of claims 1 to 5,
 - a buffer or components necessary for producing the buffer enabling binding reaction between these proteins and antibodies against HCV present in said biological sample,
 - 5 - a means for detecting the immune complexes formed in the preceding binding reaction.
17. A kit according to claim 16 wherein said at least one isolated HCV envelope protein is in an immobilised form on a solid substrate.
18. Use of an isolated HCV envelope protein according to any one of claims 1 to 5,
- 10 comprising HCV E1 protein, for *in vitro* monitoring HCV disease or prognosing the response to treatment of patients suffering from HCV infection comprising:
- incubating a biological sample from a patient with HCV infection with an E1 protein or a suitable part thereof under conditions allowing the formation of an immunological complex,
 - 15 - removing unbound components,
 - calculating the anti-E1 titers present in said sample at the start of and during the course of treatment,
 - monitoring the natural course of HCV disease, or prognosing the response to treatment of said patient on the basis of the amount of anti-E1 titers found in said sample
 - 20 at the start of treatment and/or during the course of treatment.
19. Use according to claim 18 wherein the HCV E1 protein is a HCV single E1 protein.

20. Use according to any one of claims 18 or 19 wherein said treatment is treatment with interferon.

21. A kit for monitoring HCV disease or prognosing the response to treatment of patients suffering from HCV infection comprising:

- 5 - at least one isolated HCV envelope protein according to any one of claims 1 to 5,
- a buffer or components necessary for producing the buffer enabling the binding reaction between these proteins and the anti-E1 antibodies present in a biological sample,
- means for detecting the immune complexes formed in the preceding binding
- 10 reaction, and
- optionally also an automated scanning and interpretation device for inferring a decrease of anti-E1 titers during the progression of treatment.

22. A kit according to claim 21 wherein said at least one isolated HCV envelope protein is an E1 protein.

15 23. A kit according to claim 21 or claim 22, wherein said treatment is treatment with interferon.

24. A serotyping assay for detecting one or more serological types of HCV present in a biological sample, comprising at least the following steps :

- (i) contacting the biological sample to be analyzed for the presence of HCV
- 20 antibodies of one or more serological types, with at least one isolated HCV E1 and/or E2 and/or E1/E2 protein according to any one of claims 1 to 5, under appropriate conditions which allow the formation of an immune complex,

- (ii) removing unbound components.
 - (iii) incubating the immune complexes formed with heterologous antibodies, with said heterologous antibodies being conjugated to a detectable label under appropriate conditions.
 - 5 (iv) detecting the presence of said immune complexes visually or mechanically (e.g. by means of densitometry, fluorimetry, colorimetry) and inferring the presence of one or more HCV serological types present from the observed binding pattern.
25. A serotyping assay according to claim 24 for detecting antibodies of the different
- 10 types of HCV combined in one assay format.
26. A serotyping assay according to claim 24 or claim 25 wherein said at least one isolated HCV E1 and/or E2 and/or E1/E2 protein is in an immobilised form.
27. A kit for serotyping one or more serological types of HCV present in a biological sample, comprising:
- 15 - at least one isolated HCV E1 and/or E2 and/or E1/E2 protein according to any one of claims 1 to 5,
- a buffer or components necessary for producing the buffer enabling the binding reaction between these proteins and the anti-E1 antibodies present in a biological sample.
- 20 - means for detecting the immune complexes formed in the preceding binding reaction, and

- optionally also an automated scanning and interpretation device for detecting the presence of one or more serological types present from the observed binding pattern.
28. A kit according to claim 27 for detecting antibodies to said serological types of HCV.
29. An isolated HCV envelope protein according to any one of claims 1 to 5, to raise upon immunization an E1 and/or E2 specific monoclonal antibody.
30. An isolated HCV envelope protein according to any one of claims 1 to 5, for the preparation of an immunoassay kit.
31. Use of an isolated HCV envelope protein according to any one of claims 1 to 5, for detecting HCV antibodies present in a biological sample.
32. Use of an isolated HCV envelope protein according to any one of claims 1 to 5, for the manufacture of a medicament for immunising a mammal against HCV.
33. Use according to claim 32 wherein said mammal is human.
34. An isolated HCV single or specific oligomeric envelope protein, substantially as herein described with reference to one or more of the examples but excluding comparative examples.
35. A method for immunising a mammal against HCV, substantially as herein described with reference to one or more of the examples but excluding comparative examples.
36. A vaccine composition for immunising a mammal against HCV, substantially as herein described with reference to one or more of the examples but excluding comparative examples.

37. A method for *in vitro* diagnosis of HCV antibodies present in a biological sample, substantially as herein described with reference to one or more of the examples but excluding comparative examples.
38. A kit for determining the presence of HCV antibodies present in a biological
5 sample, substantially as herein described with reference to one or more of the examples but excluding comparative examples.
39. Use of an isolated HCV single or specific oligomeric envelope protein, substantially as herein described with reference to one or more of the examples but excluding comparative examples.
- 10 40. A kit for monitoring HCV disease or prognosing the response to treatment of patients suffering from HCV infection, substantially as herein described with reference to one or more of the examples but excluding comparative examples.
41. A serotyping assay for detecting one or more serological types of HCV present in a biological sample, substantially as herein described with reference to one or more of the
15 examples but excluding comparative examples.
42. A kit for serotyping one or more serological types of HCV present in a biological sample, substantially as herein described with reference to one or more of the examples but excluding comparative examples.

DATED this 29th Day of October, 1999

INNOGENETICS N.V.

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Fellow Institute of Patent and Trade Mark Attorneys of
Australia of BALDWIN SHELSTON WATERS

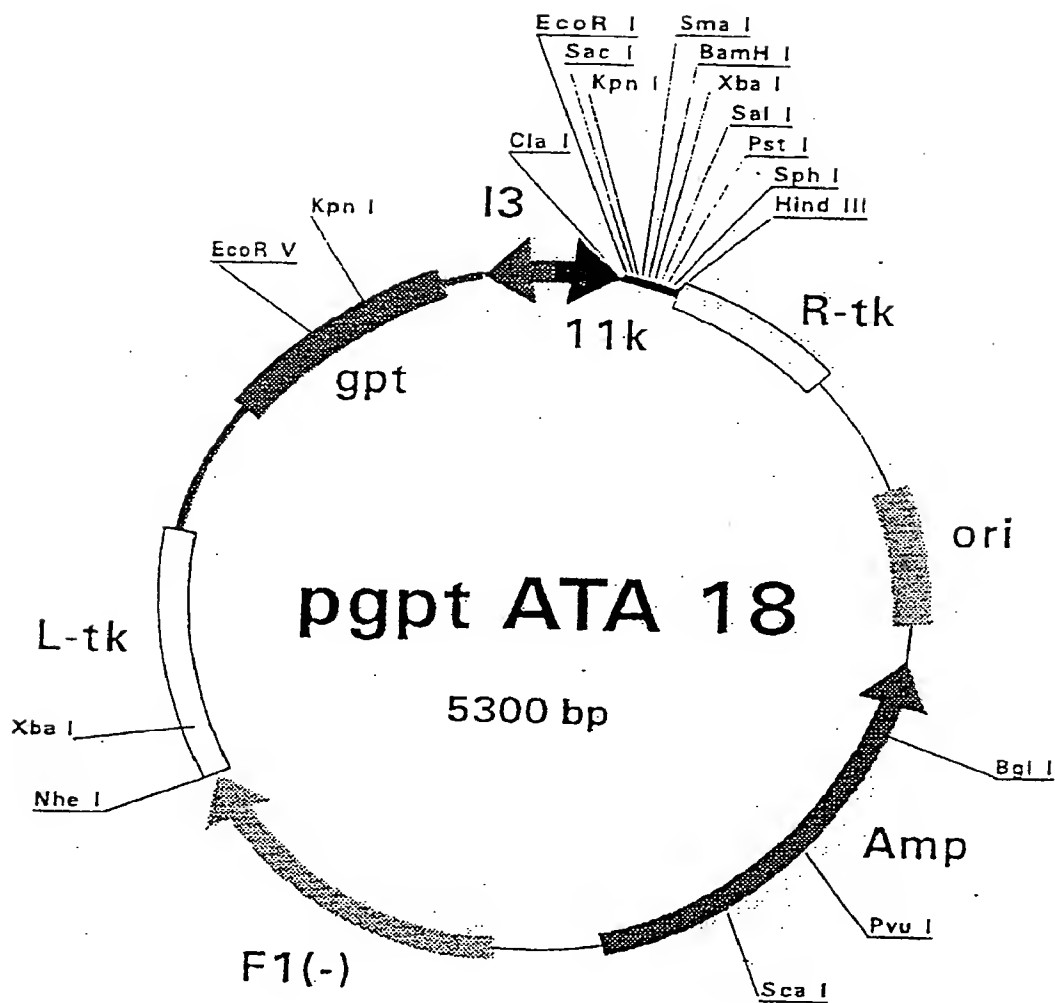


FIGURE 1

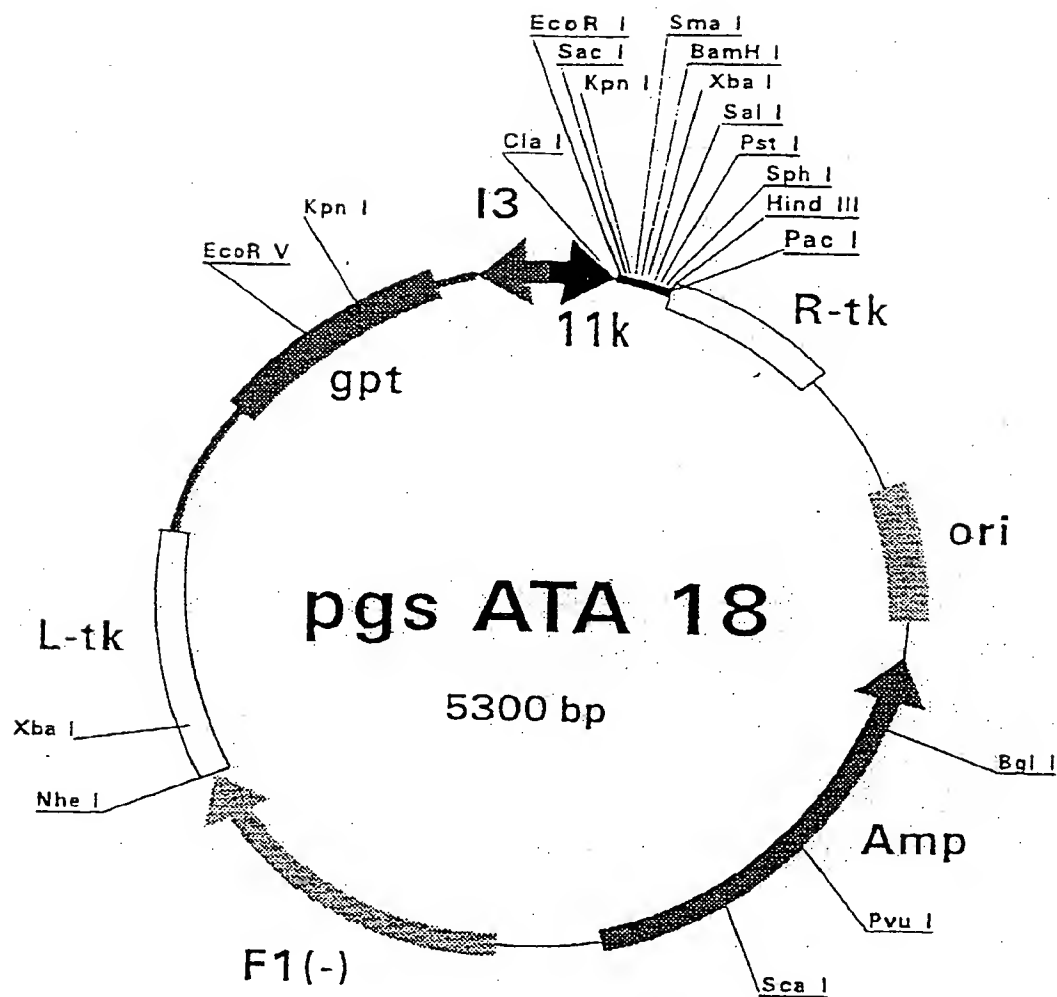


FIGURE 2

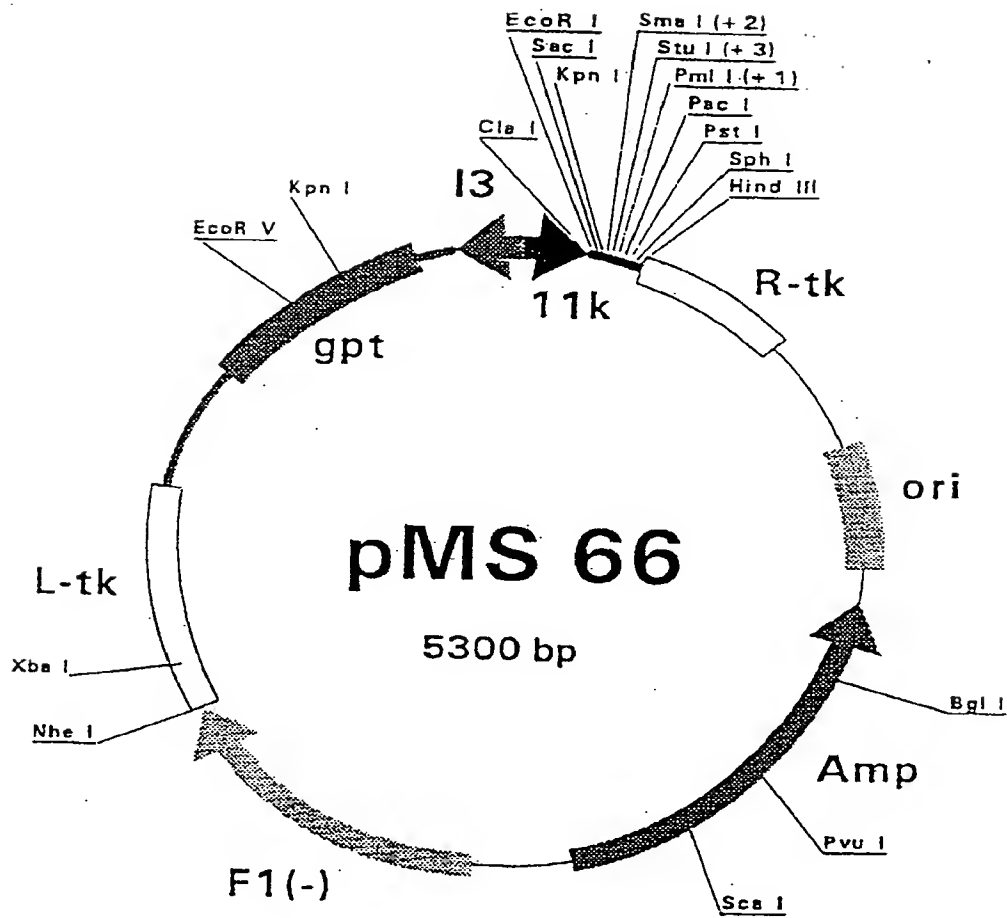


FIGURE 3

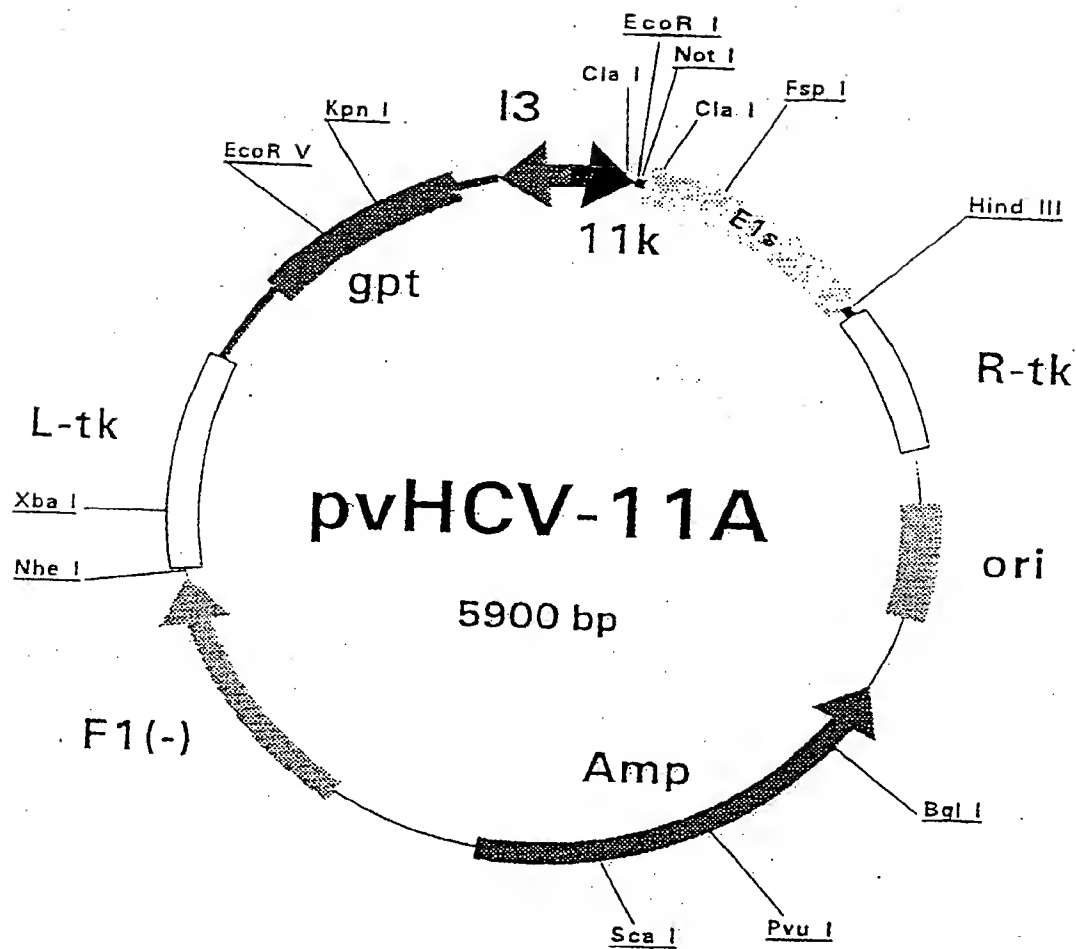
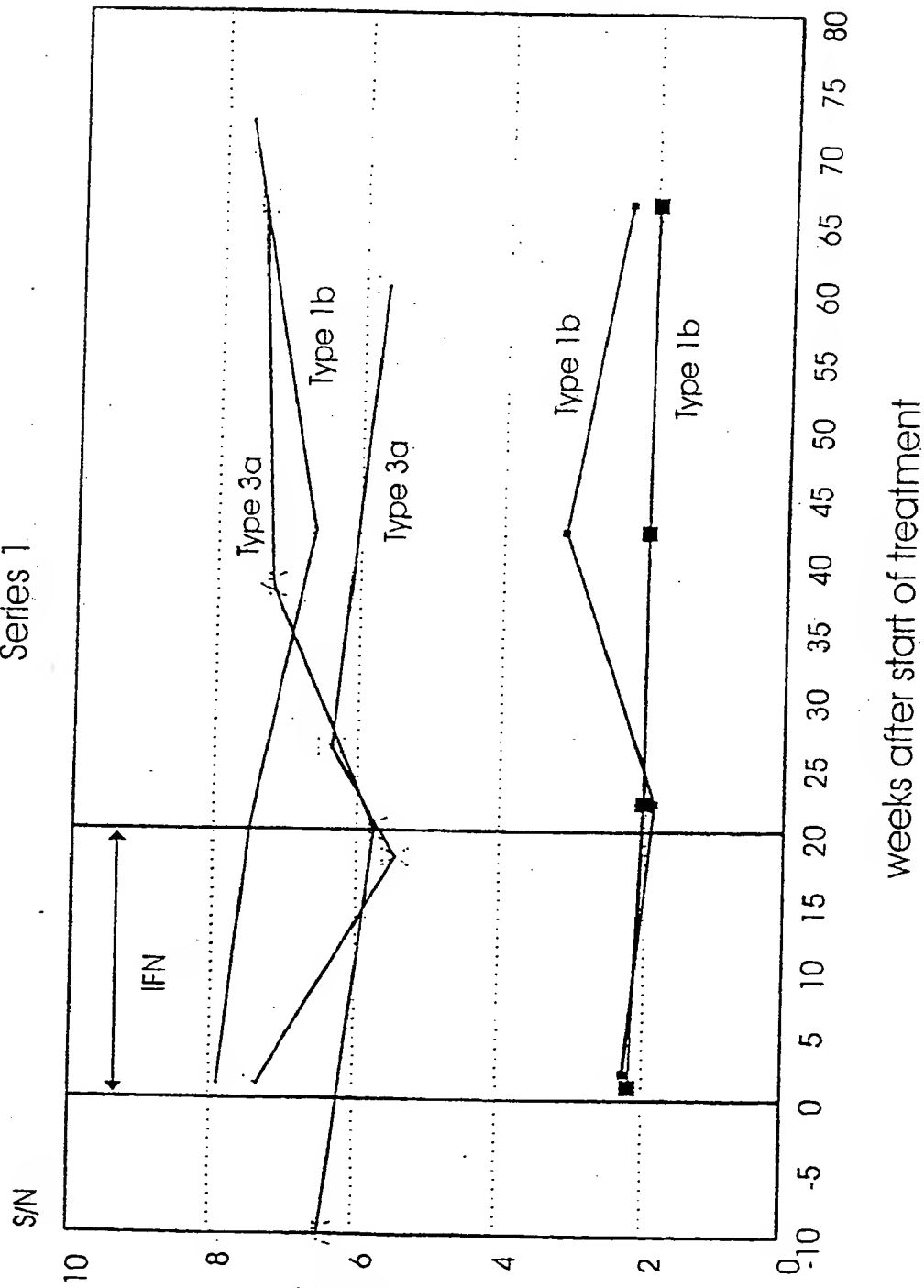


FIGURE 4

09 10 99 5:12 PM

Anti-E1 levels in NON-responders to IFN treatment

Series 1



5/59

FIGURE 5

00 10 00 57007

Anti-E1 levels in RESPONDERS to IFN treatment

SERIES 1

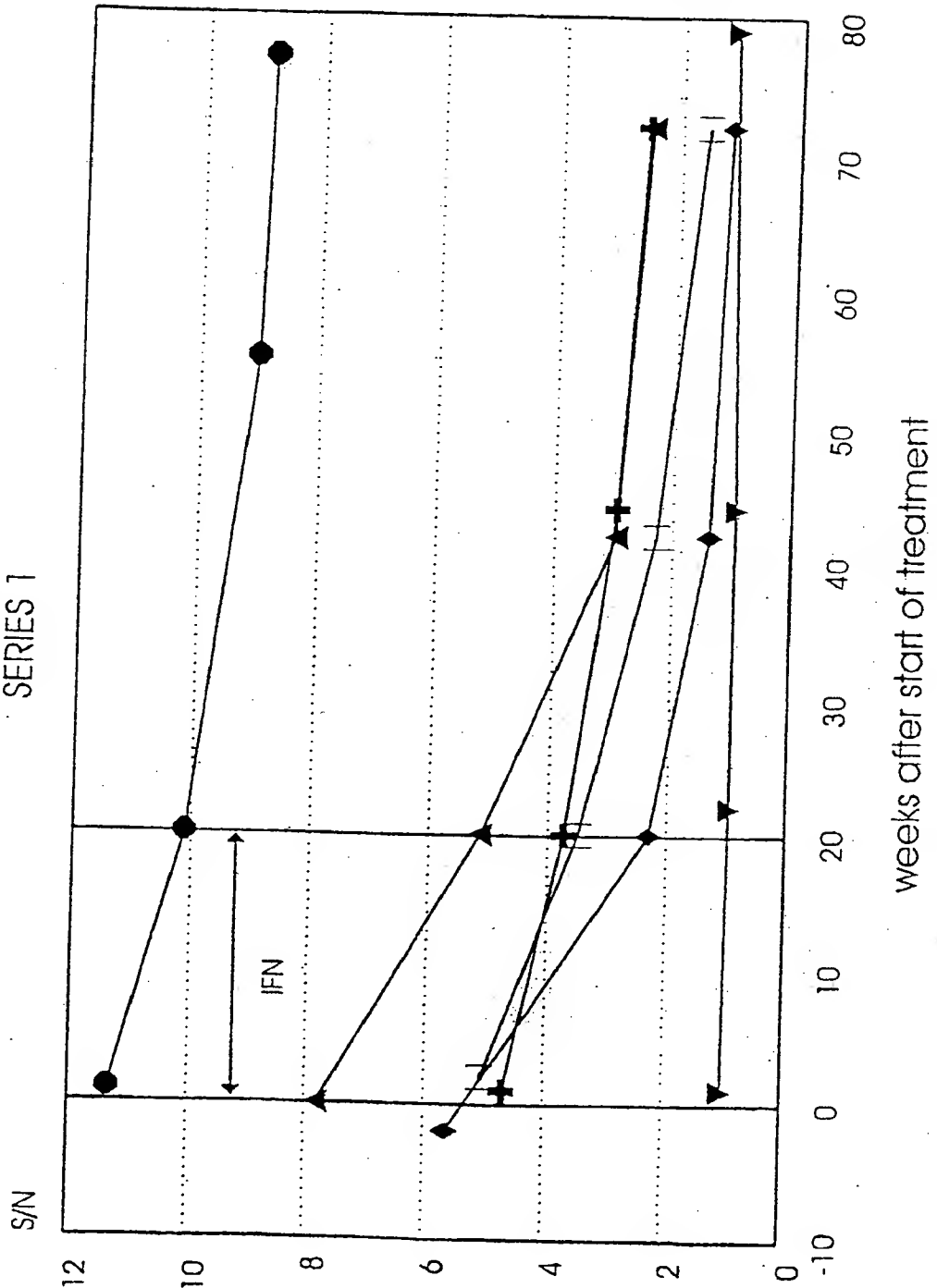
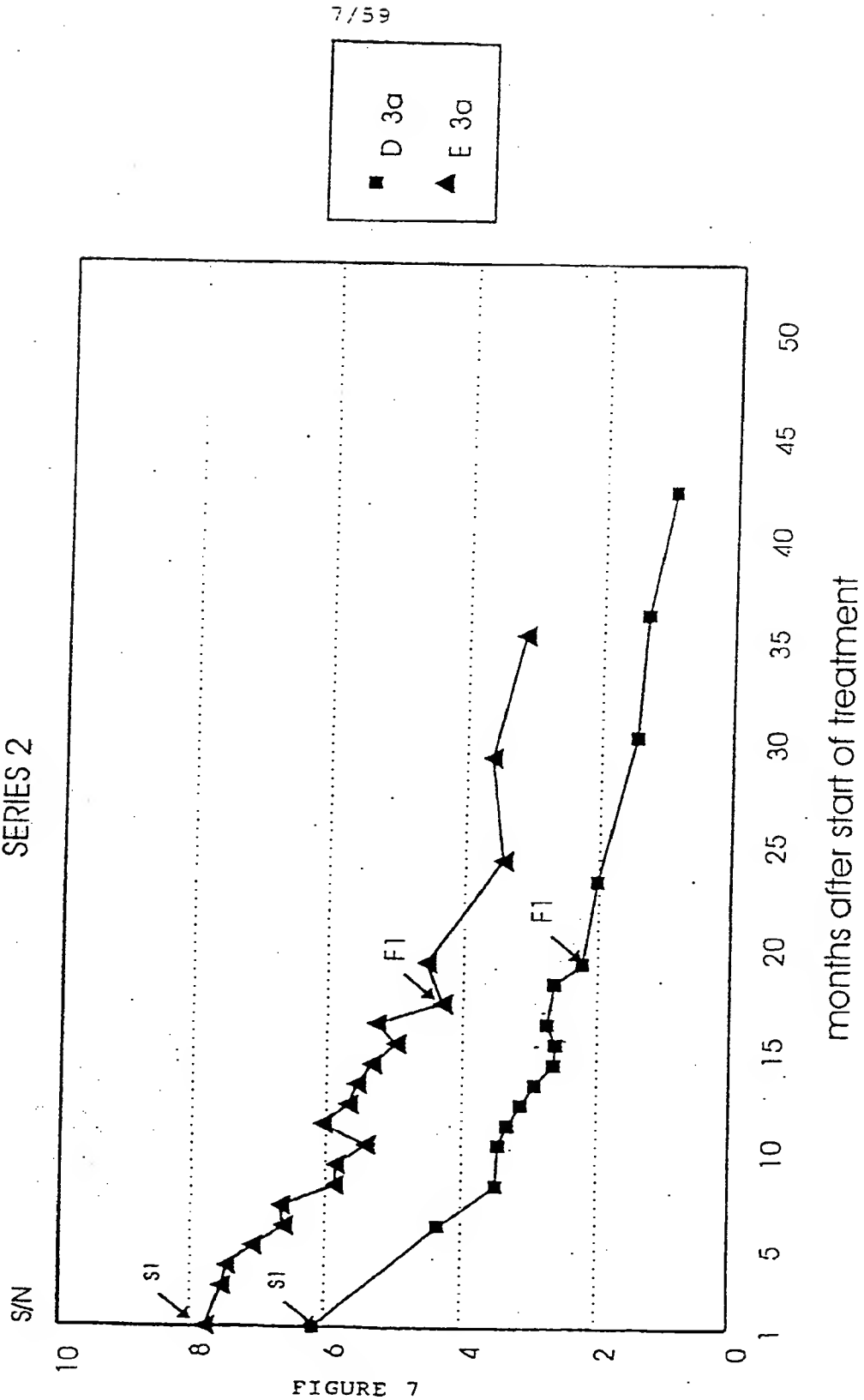


FIGURE 6

29 10 88 37127

Anti-E1 levels in patients with COMPLETE response to IFN

SERIES 2



09 10 99 37107

8/59

Anti-E1 levels in INCOMPLETE responders to IFN treatment SERIES 2

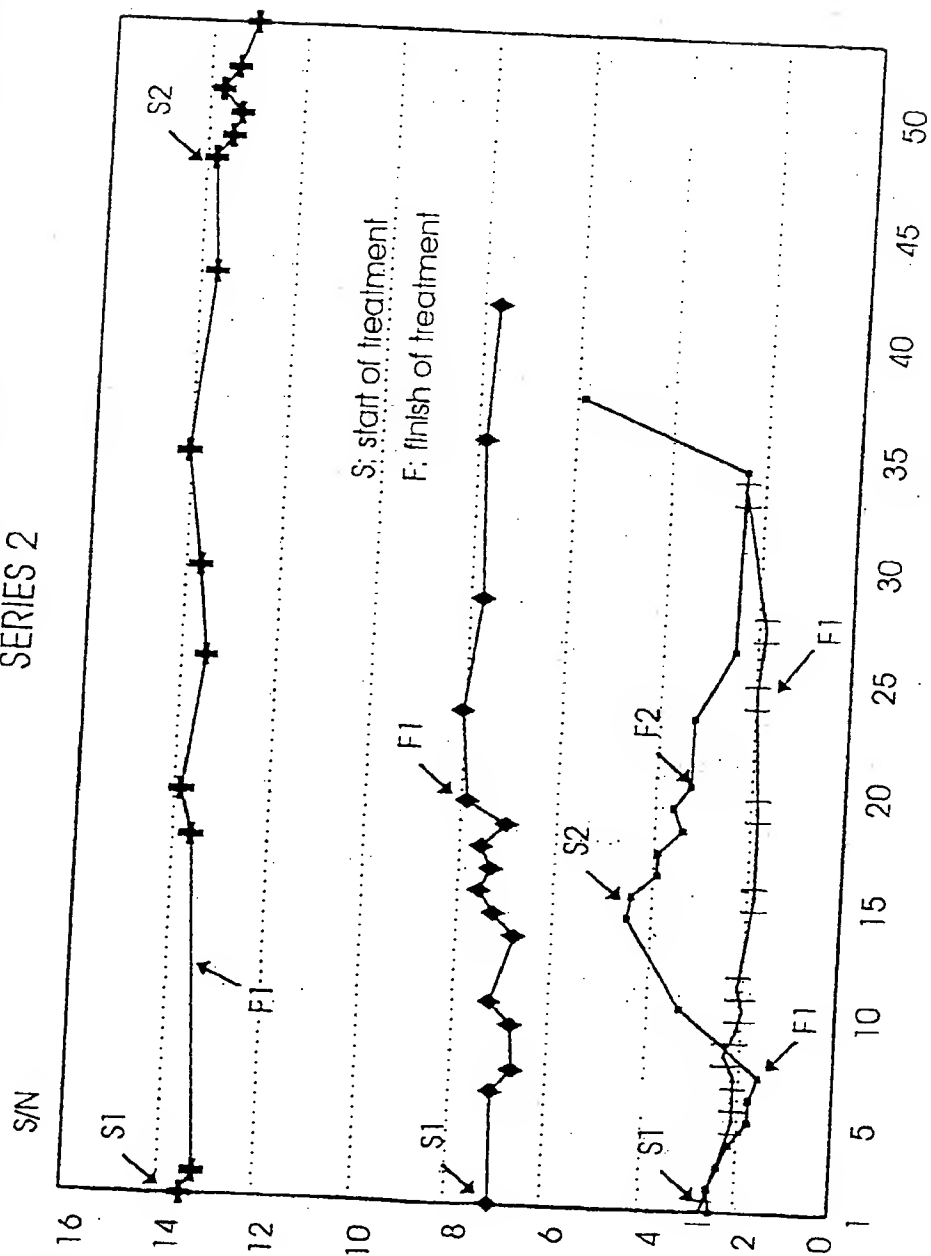


FIGURE 8

months after start of treatment

09 10 00 57107

Anti-E2 levels in NON-RESPONDERS to IFN treatment

SERIES 1

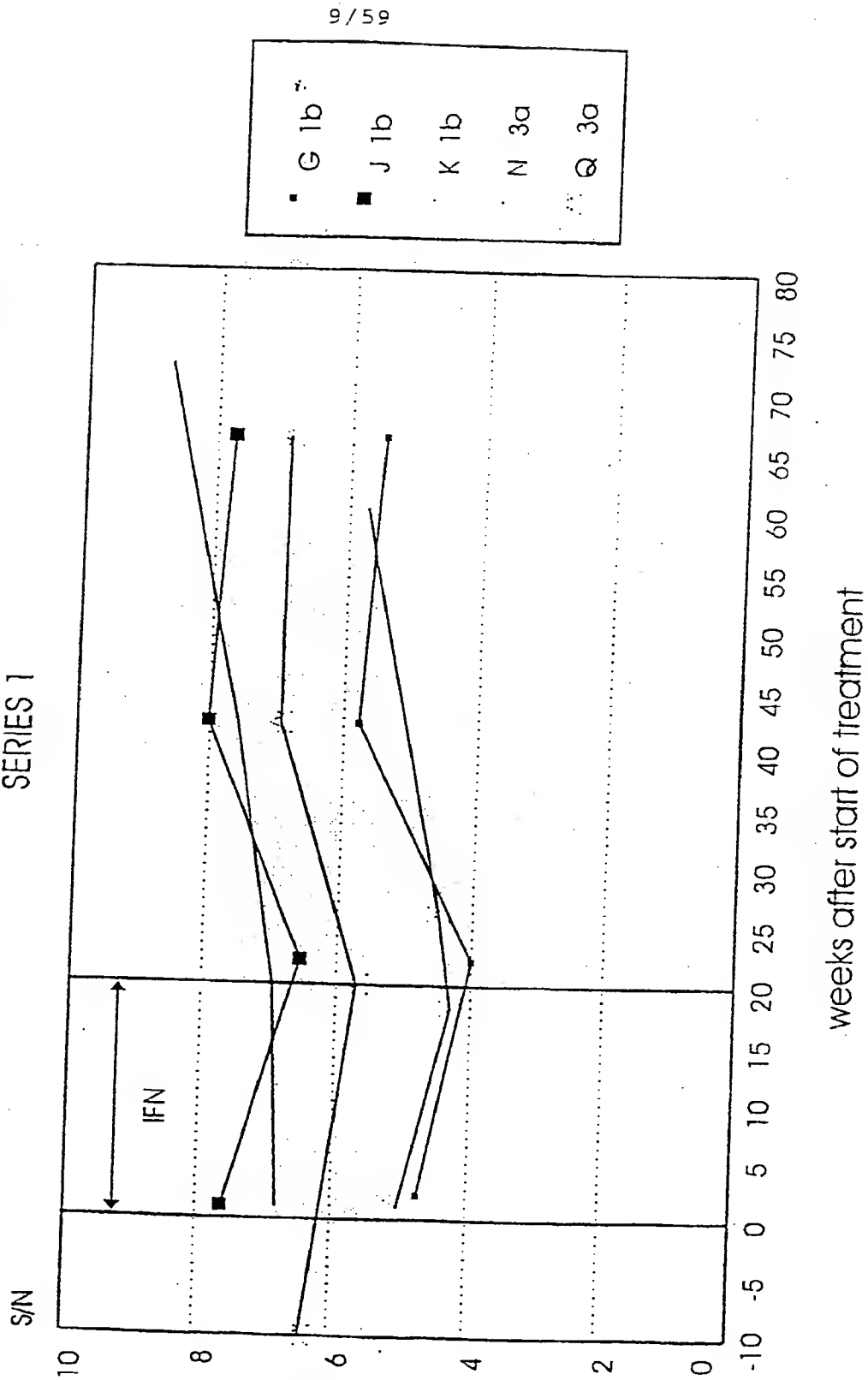
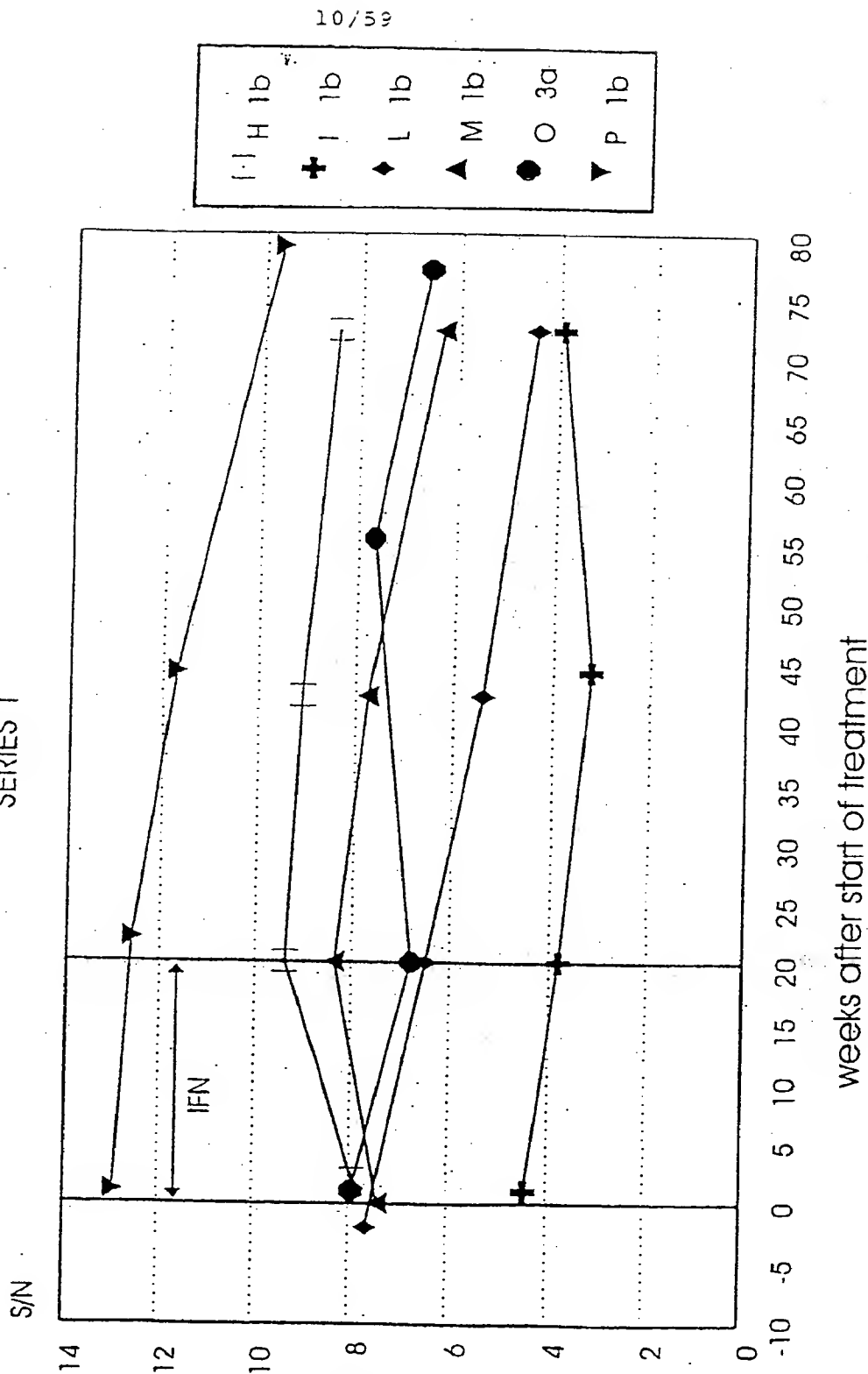


FIGURE 9

33 10 30 57.23

Anti-E2 levels in RESPONDERS to IFN treatment

SERIES 1



09 10 00 57127

Anti-E2 levels in INCOMPLETE responders to IFN treatment

SERIES 2

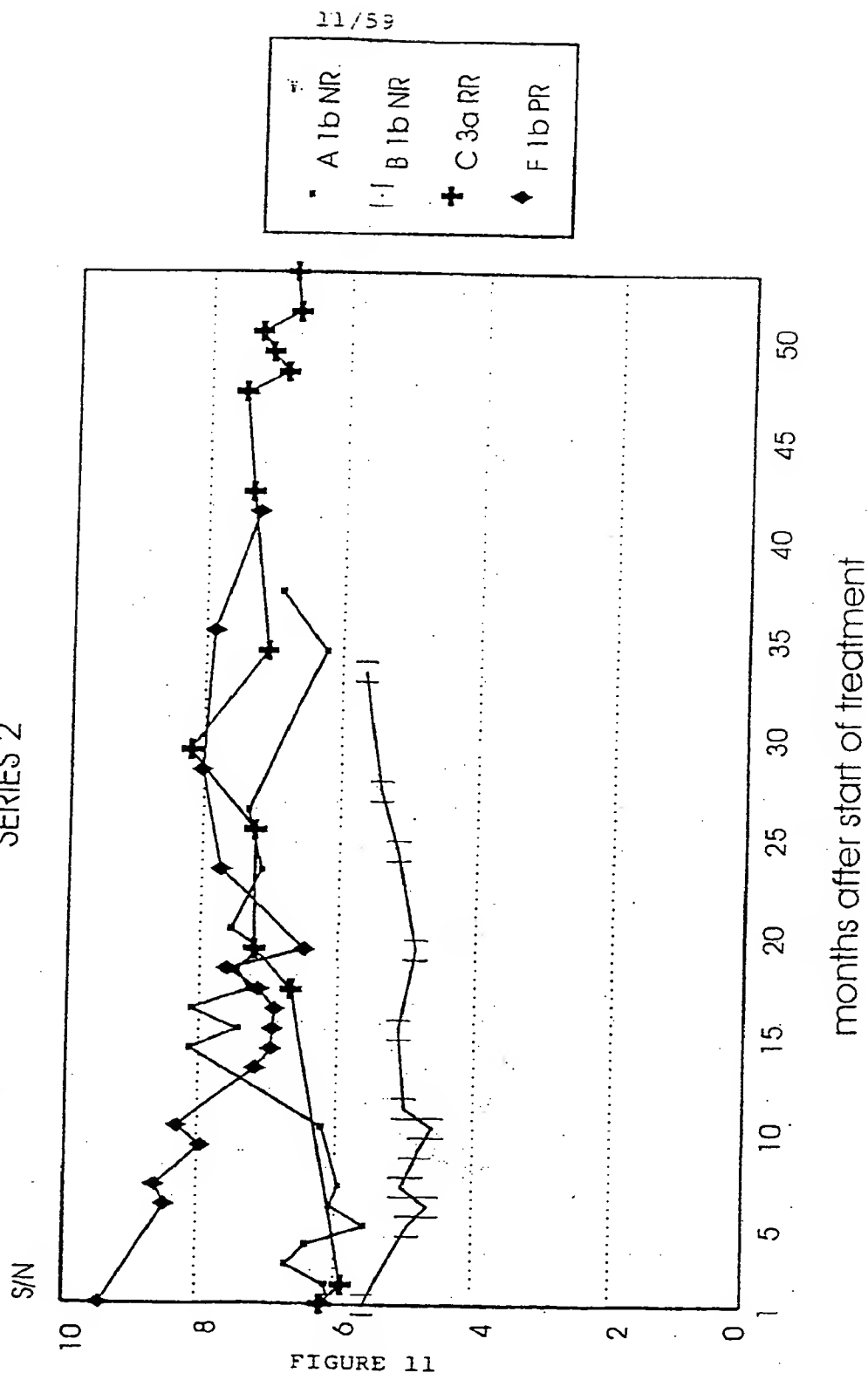


FIGURE 11

29 10 99 37:23

Anti-E2 levels in COMPLETE responders to IFN treatment

SERIES 2

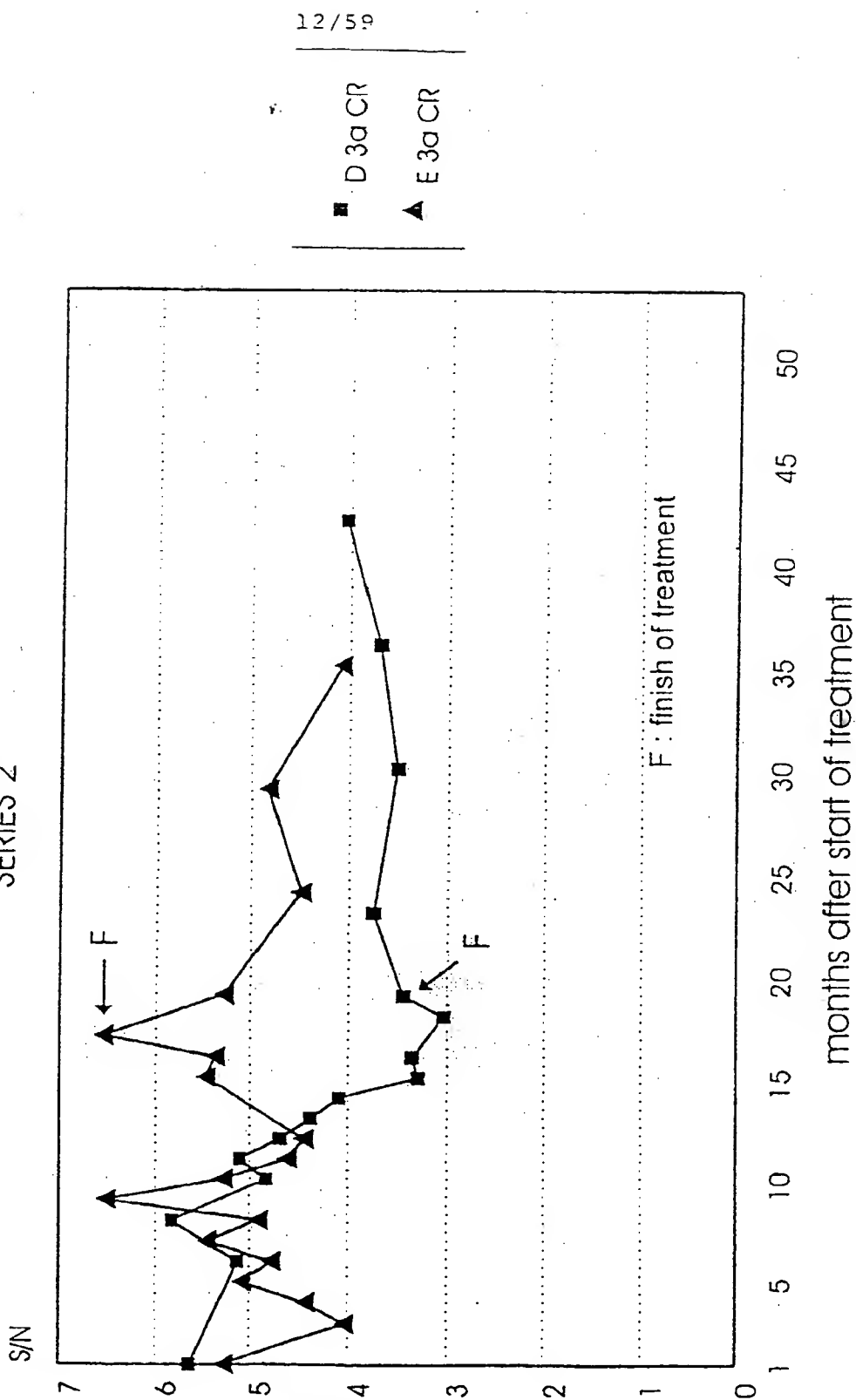


FIGURE 12

FIGURE 13

Human anti-E1 reactivity competed with peptides

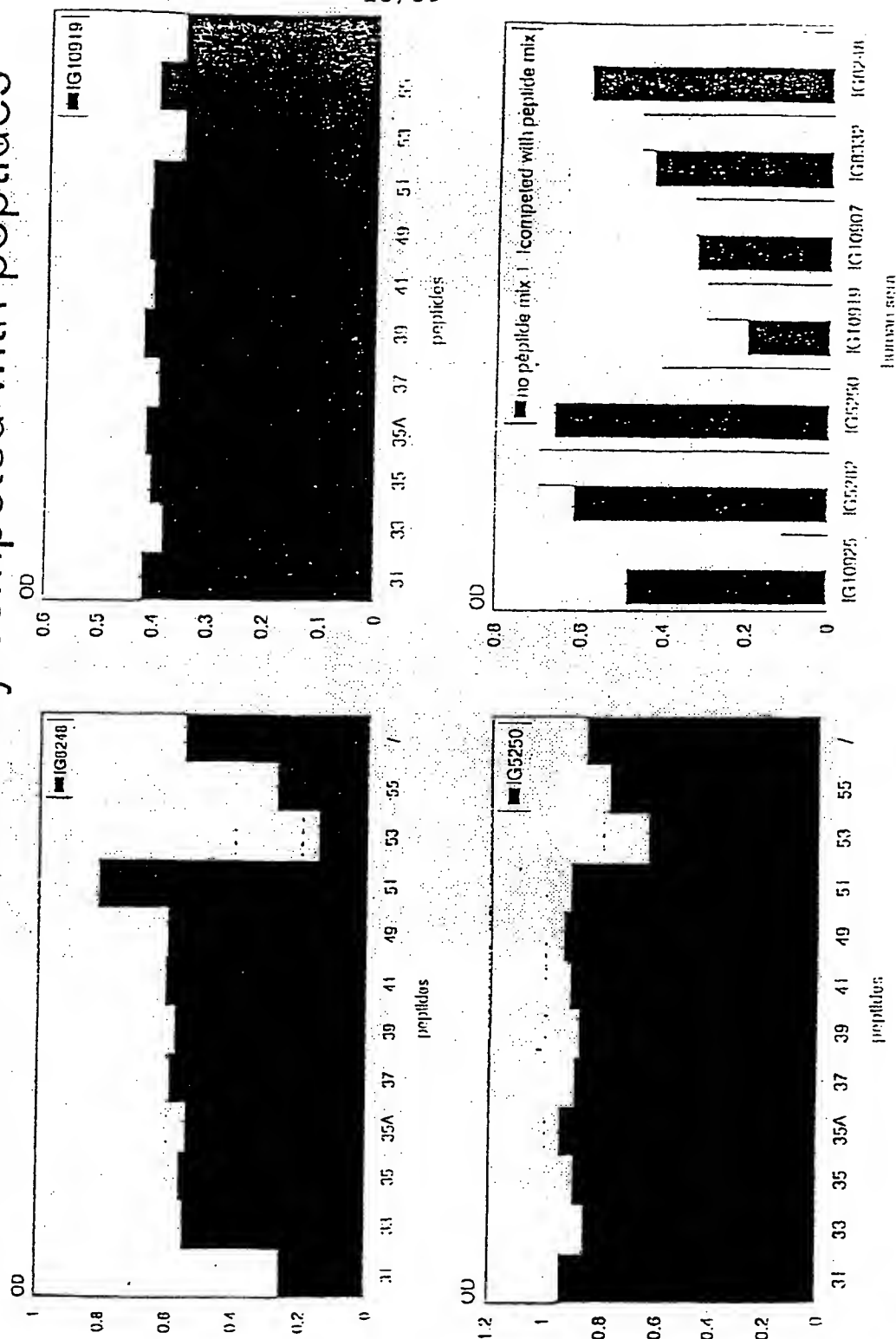
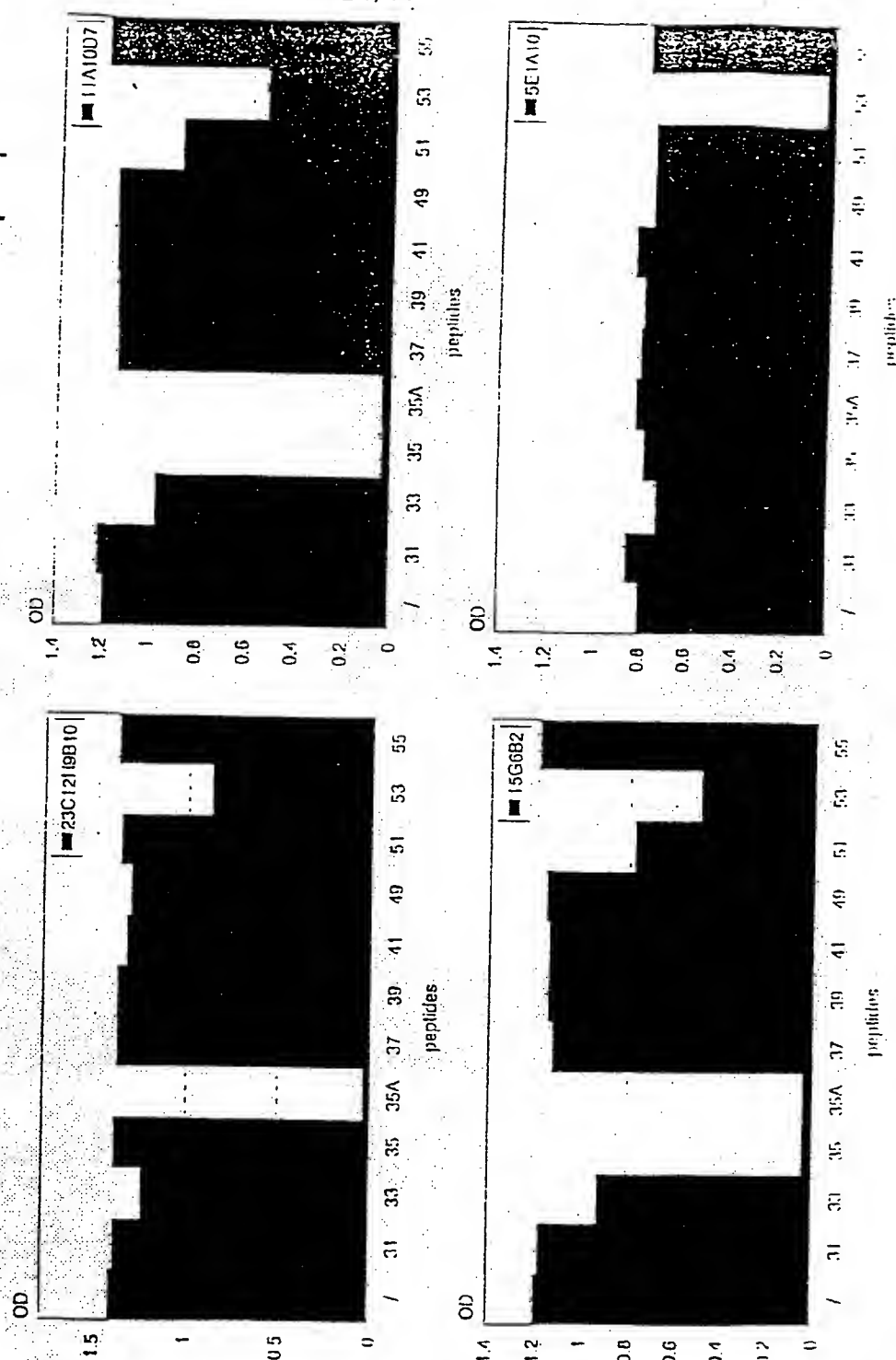


FIGURE 14

Competition of reactivity of anti-E1 Mabs with peptides



29 10 88 5:02:37

Anti-E1 (epitope 1) levels in NON-RESPONDERS to IFN treatment

SERIES 1

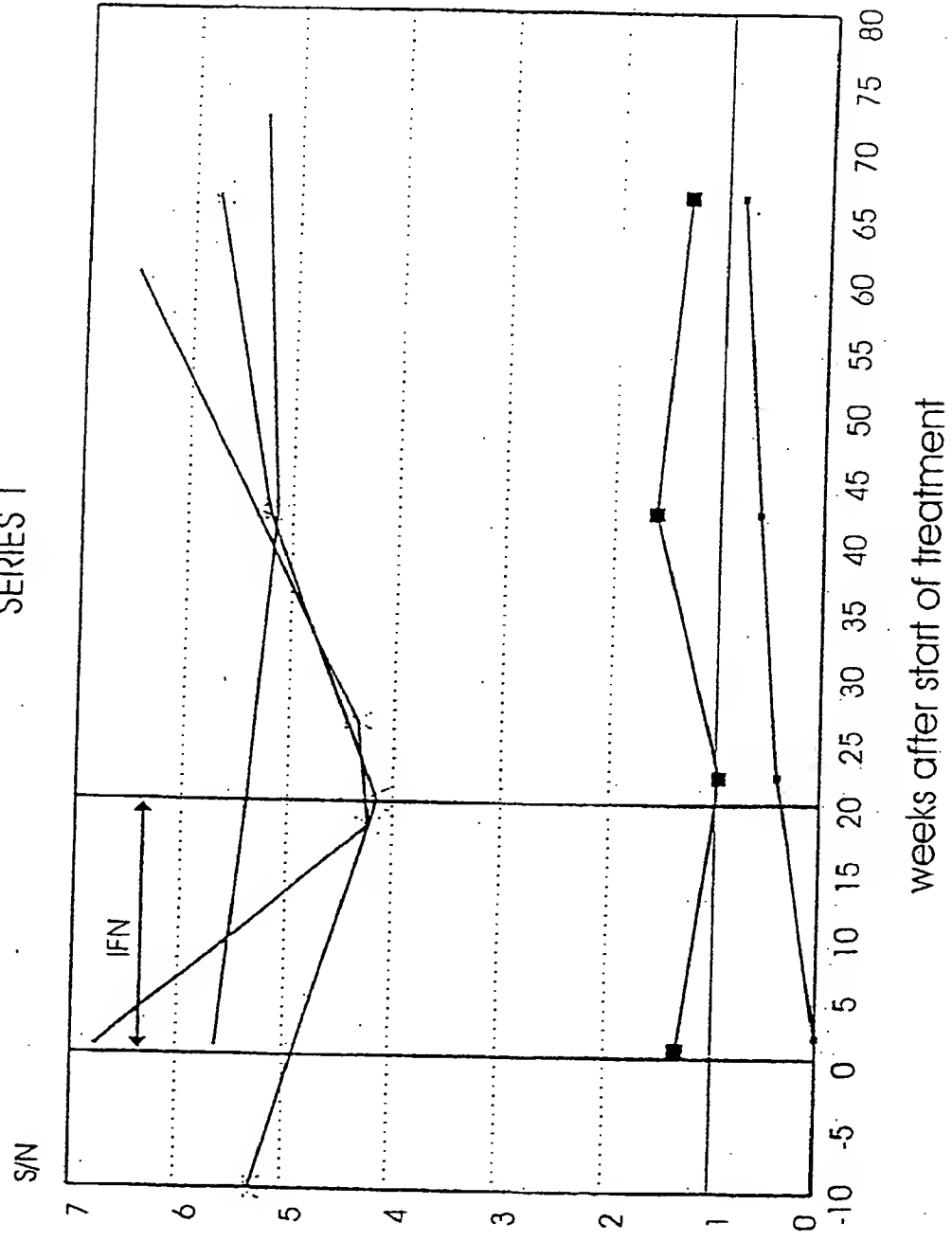


FIGURE 15

09 10 88 5003

Anti-E7 (epitope 1) levels in RESPONDERS to IFN treatment

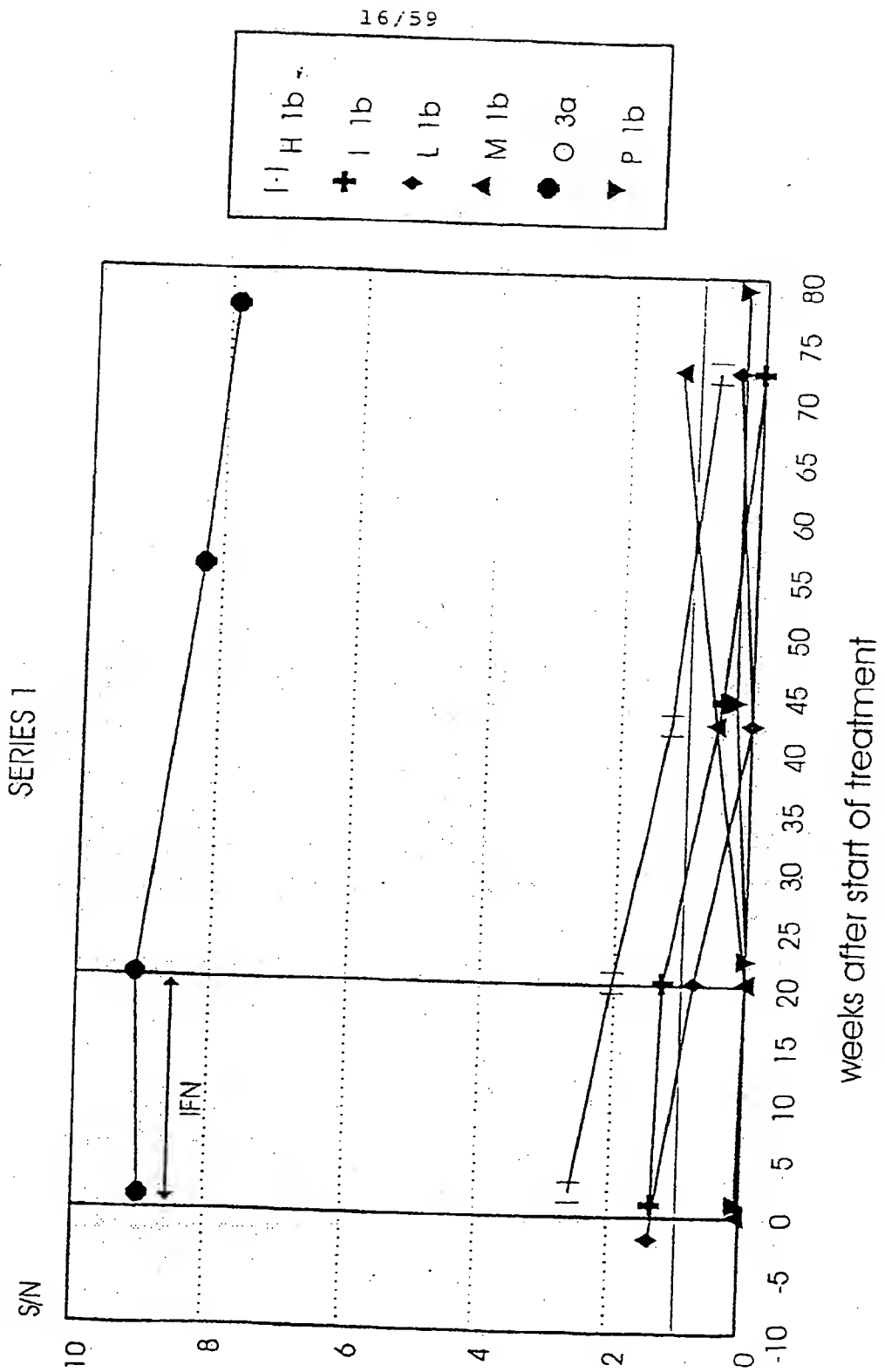


FIGURE 16

09 10 00 57:27

Anti-E1 (epitope 2) levels in NON-RESPONDERS to IFN treatment

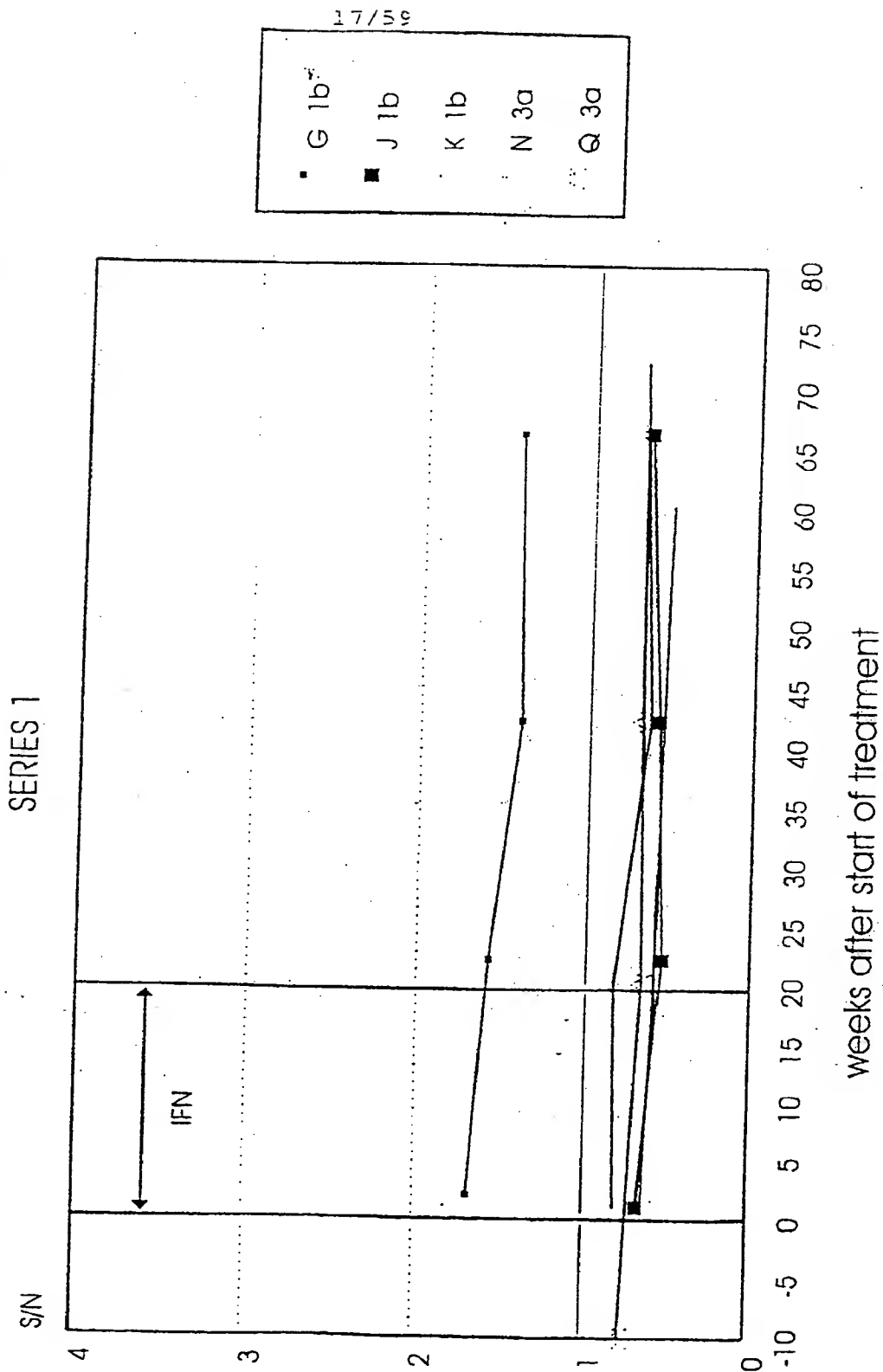


FIGURE 17

00 10 00 57107

Anti-E1 (epitope 2) levels in RESPONDERS to IFN treatment

SERIES 1

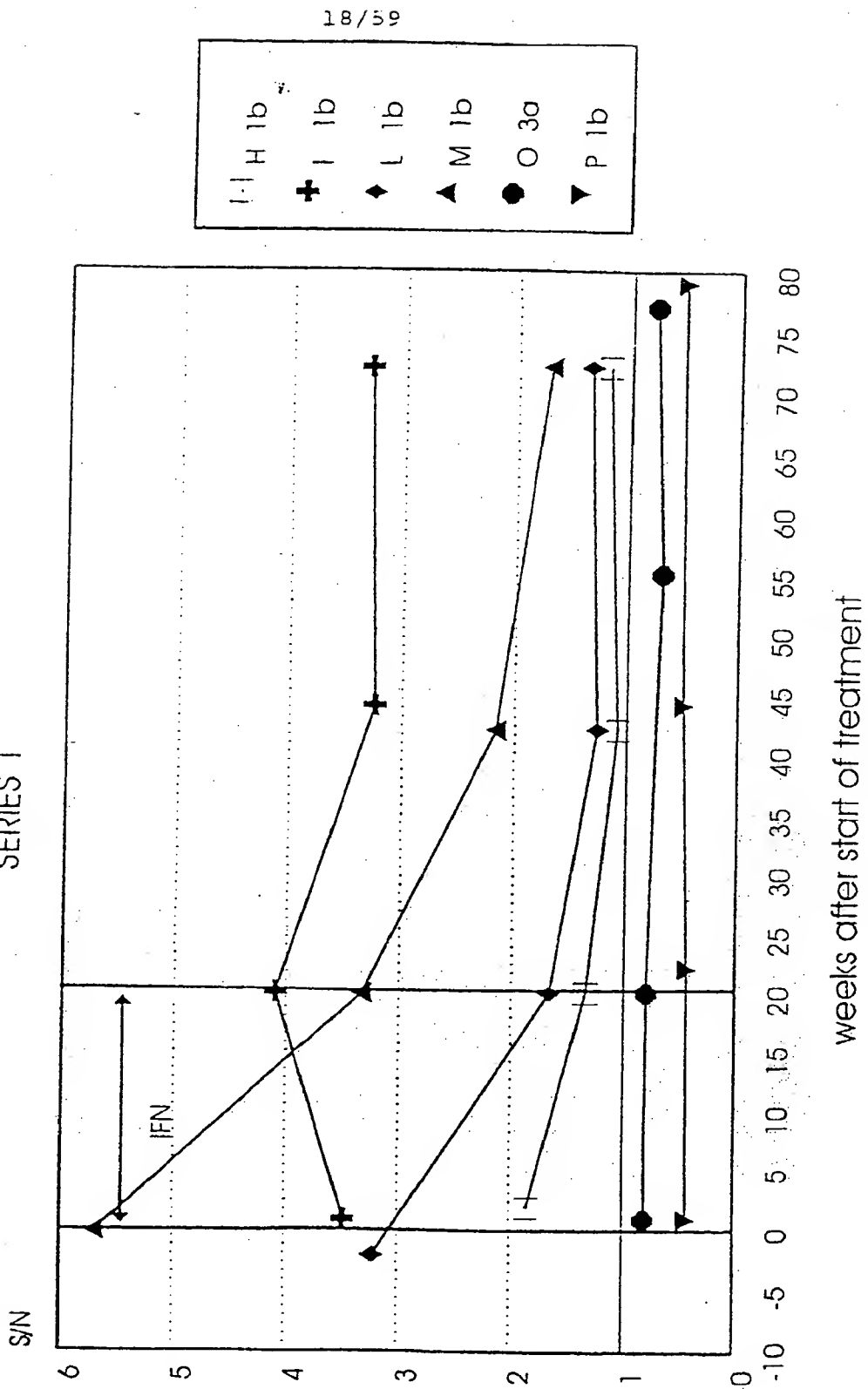
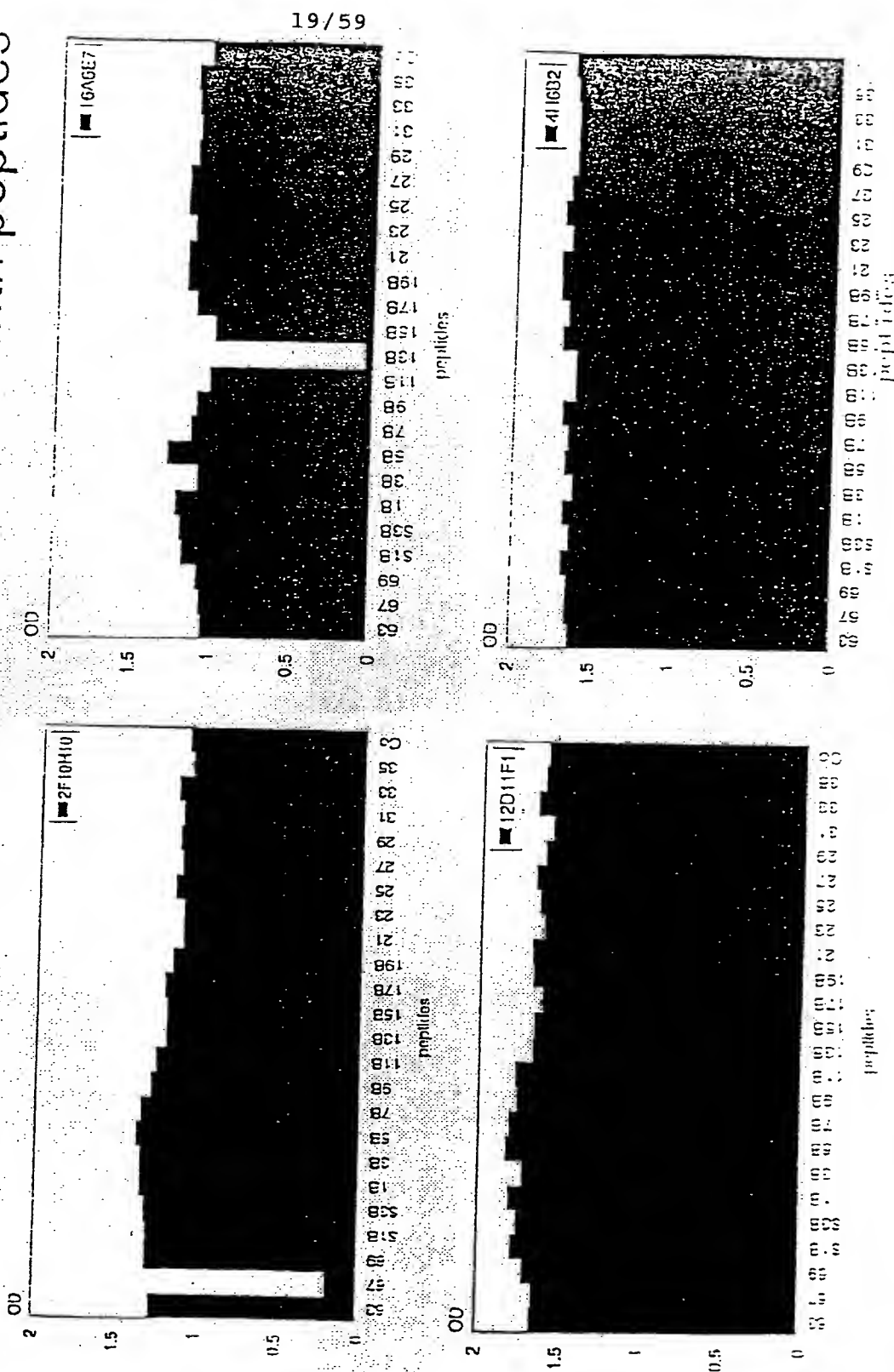


FIGURE 18

FIGURE 19

Competition of reactivity of anti-E2 Mabs with peptides



0010005737

FIGURE 20

Human anti-E2 reactivity competed with peptides

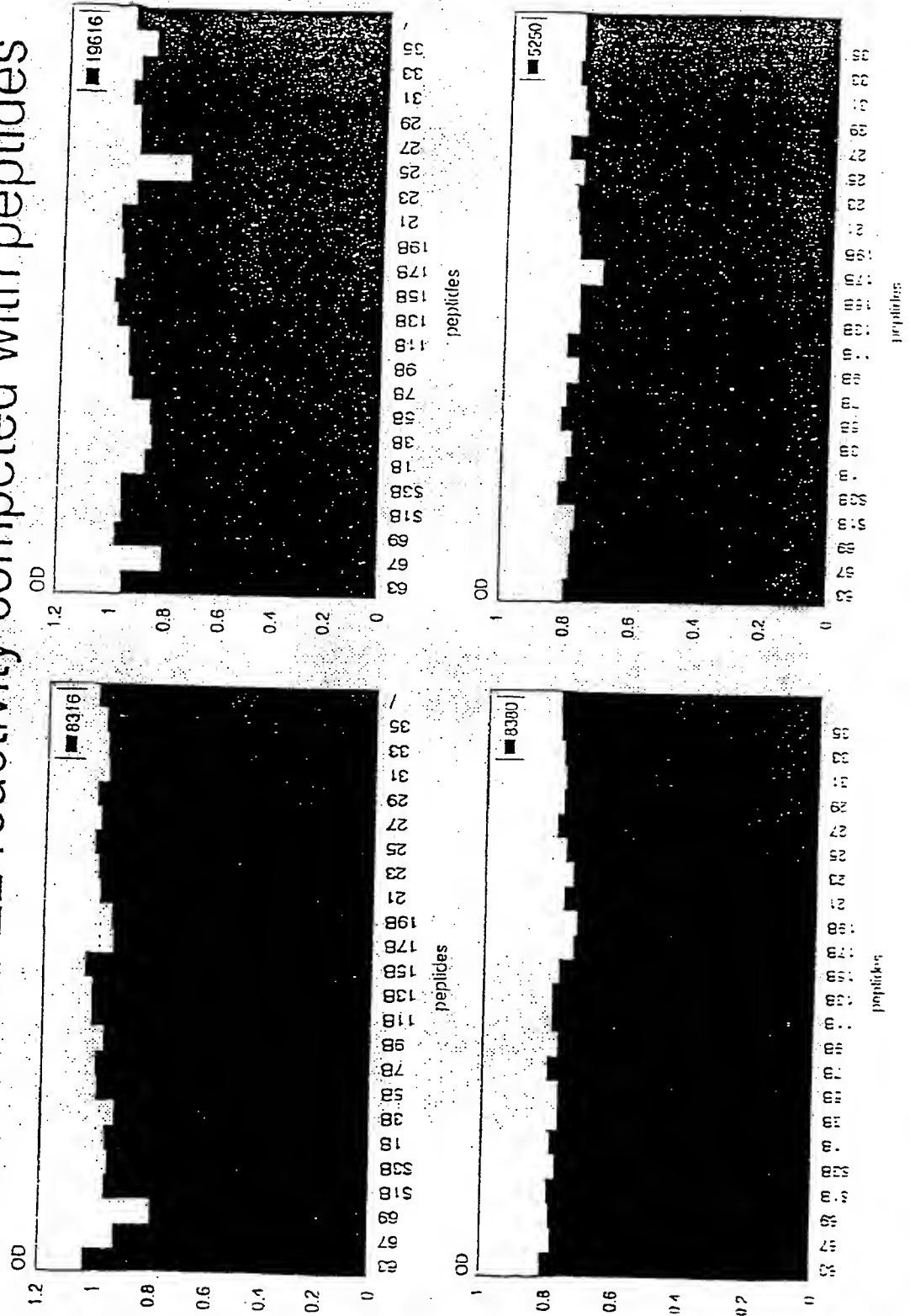


Figure 21

5' GGCATGCAAGCTTAATTAATT3' (SEQ ID NO 1)
3'ACGTCCGTACGTTTCAATTAATTAATCGA5' (SEQ ID NO 94)

5'CCGGGGAGGCCTGCACGTGATCGAGGGCAGACACCATCACCACCATCACTAATAGT
TAATTAAGTCA 3' (SEQ ID NO 2)
3'CCTCCGGACGTGCACTAGCTCCCGTCTGTGGTAGTGGTGGTAGTGATTATCAATTAATTG
5' (SEQ ID NO 95)

SEQ ID NO 3 (HCC19A)
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CCAAGTCAAGCATTGTGTATGAGGCAGCGGACATGATCATGCACACCCCCGGGTGCGT
GCCCTGCGTTCCGGGAGAACAACCTCTCCCGCTGCTGGGTAGCGCTCACCCCCACGCTC
GCAGCTAGGAACGCCAGCGTCCCCACCACGACAATACGACGCCACGTCGATTTGCTCG
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CTCGTCTCCAGCTGTTACCATCTCGCCTCGCCGGCATGAGACGGTGCAGGACTGCA
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GAACTGGTCGCCTACAACGGCCCTGGTGGTATCGCAGCTGCTCCGGATCCCACAAGCT
GTCGTGGACATGGTGGCGGGGGCCATTGGGGAGTCTTGGCGGGCCTCGCCTACTATT
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SEQ ID NO 5 (HCC110A)
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ATCTTCCTCTTGGCTTTGCTGTCTGTCTGACCGTTCCAGCTTCCGCTTATGAAGTGCG
CAACGTGTCCGGGATGTACCATGTACGAACGACTGCTCCAAGTCAAGCATTGTGTAT
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SEQ ID NO 7 (HCCI11A)

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TCTTCCTCTTGGCTTTACTGTCTGTCTGACCATTCAGCTTCCGCTTATGAGGTGCGC
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SEQ ID NO 16 (HCP52)

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SEQ ID NO 17 (HCP53)

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SEQ ID NO 18 (HCP54)

CTATTACCAGTTCATCATCATATCCCA

SEQ ID NO 19 (HCP107)

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SEQ ID NO 20 (HCP108)

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SEQ ID NO 29 (HCC162)

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SEQ ID NO 31 (HCCI63)

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CCTTGAGGACGGGGTAAACTATGCAACAGGGAATTTACCGGTTGCTCTTTCTCTATCT
TTATTCTTGCTCTTCTCTCGTGTCTGACCGTTCCGGCCTCTGCAGTTCCTACCGAAATG
CCTCTGGGATTTATCATGTTACCAATGATTGCCCAAACCTCTCCATAGTCTATGAGGCA
GATAACCTGATCCTACACGCACCTGGTTGCGTGCCTTGTGTCTATGACAGGTAATGTGA
GTAGATGCTGGGTCCAAATTACCCCTACACTGTCAGCCCCGAGCCTCGGAGCAGTCAC
GGCTCCTCTTCGGAGAGCCGTTGACTACCTAGCGGGAGGGGCTGCCCTCTGCTCCGCG
TTATACGTAGGAGACGCGTGTGGGGCACTATTCTTGGTAGGCCAAATGTTACCTATA
GGCCTCGCCAGCACGCTACGGTGCAGAACTGCAACTGTTCCATTTACAGTGGCCATGT
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SEQ ID NO 33 (HCP109)

TGGGATATGATGATGAACTGGTC

SEQ ID NO 34 (HCP72)

CTATTATGGTGGTAAKGCCARCARGAGCAGGAG

SEQ ID NO 35 (HCCL22A)

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GCCTCGCCTACTATTCCATGGTGGGGAACCTGGGCTAAGGTTTTGGTTGTGATGCTACTC
TTTGCCGGCGTCGACGGGCATACCCGCGTGTGAGGAGGGGCGAGCAGCCTCCGATACCA
GGGGCCTTGTGTCCCTCTTTAGCCCCGGGTGCGGCTCAGAAAATCCAGCTCGTAAACAC
CAACGGCAGTTGGCACATCAACAGGACTGCCCTGAACTGCAACGACTCCCTCCAAAC
AGGGTTCTTTGCCGCACTATTCTACAAACACAAATTCAACTCGTCTGGATGCCAGAG
CGCTTGGCCAGCTGTGCTCCATCGACAAGTTGCTCAGGGGTGGGGTCCCCTCACTT
AACTGAGCCTAACAGCTCGGACCAGAGGCCCTACTGCTGGCACTACGCGCCTCGACC
GTGTGGTATTGTACCCGCGTCTCAGGTGTGCGGTCCAGTGTATTGCTTACCCCGAGCC
CTGTTGTGGTGGGGACGACCGATCGGTTTGGTGTCCCCACGTATAACTGGGGGGCGAA
CGACTCGGATGTGCTGATTCTCAACAACACGCGGCCCGCGAGGCAACTGGTTCGGC
TGTACATGGATGAATGGCACTGGGTTTACCAAGACGTGTGGGGGGCCCCCGTGAACA
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CGAGGCCACCTACGCCAGATGCGGTTCTGGGCCCTGGCTGACACCTAGGTGTATGTT

CATTACCCATATAGGCTCTGGCACTACCCCTGCACTGTCAACTTCACCATCTTCAAGGT
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GCCGCTTAGAGAACCTGGTGGTCTCAATGCGGCGGCCGTGGCCGGGGCGCATGGC
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SEQ ID NO 37 (HCC141)

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GGGGCCTTGTGTCCCTCTTTAGCCCCGGGTGGCTCAGAAAATCCAGCTCGTAAACAC
CAACGGCAGTTGGCACATCAACAGGACTGCCCTGAACTGCAACGACTCCCTCCAAAC
AGGGTTCTTTGCCGCACTATTCTACAAACACAAATTCAACTCGTCTGGATGCCAGAG
CGCTTGGCCAGCTGTGCTCCATCGACAAGTTGCTCAGGGGTGGGGTCCCTCACTT
ACACTGAGCCTAACAGCTCGGACCAGAGGCCCTACTGCTGGCACTACGCGCCTCGACC
GTGTGGTATTGTACCCGCGTCTCAGGTGTGCGGTCCAGTGTATTGCTTCACCCGAGCC
CTGTTGTGGTGGGGACGACCGATCGGTTTGGTGTCCCCACGTATAACTGGGGGGCGAA
CGACTCGGATGTGCTGATTCTCAACAACACGCGGCCCGCCGCGAGGCAACTGGTTCGGC
TGTACATGGATGAATGGCACTGGGTTACCAAGACGTGTGGGGGCCCGCGTGAACA
TCGGGGGGGGCCGGCAACAACACCTTGACCTGCCCCACTGACTGTTTTCGGAAGCACCC
CGAGGCCACCTACGCCAGATGCGGTTCTGGGCCCTGGCTGACACCTAGGTGTATGGTT
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TAGGATGTACGTGGGGGGCGTGGAGCACAGGTTTGAAGCCGCATGCAATTGGACTCG
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SEQ ID NO 39 (HCC142)

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CCTCGCCTACTATTCCATGGTGGGGAACCTGGGCTAAGGTTTTGGTTGTGATGCTACTCT

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SEQ ID NO 41 (HCC143)

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CGTCTCAGGTGTGCGGTCCAGTGTATTGCTTACCCCCGAGCCCTGTTGTGGTGGGGAC
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SEQ ID NO 43 (HCCI44)

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SEQ ID NO 45 (HCCL64)

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SEQ ID NO 47 (HCC165)

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SEQ ID NO 49 (HCC166)

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TGTGGCCGCTGCTCCTGCTTCTGCTGGCCTTACCACCACGAGCTTATGCCTAGTAA

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Figure 22

OD measured at 450 nm
construct

| Fraction | volume | dilution | 39 Type 1b | 40 Type 1b | 62 Type 3a | 63 Type 5a |
|--------------|--------|----------|------------------|------------------|------------------|------------------|
| START | 23 ml | 1/20 | 2.517 | 1.954 | 1.426 | 1.142 |
| FLOW THROUGH | 23 ml | 1/20 | 0.087 | 0.085 | 0.176 | 0.120 |
| 1 | 0.4 ml | 1/200 | 0.102 | 0.051 | 0.048 | 0.050 |
| 2 | | | 0.396 | 0.550 | 0.090 | 0.067 |
| 3 | | | 2.627 | 2.603 | 2.481 | 2.372 |
| 4 | | | 3 | 2.967 | 3 | 2.694 |
| 5 | | | 3 | 2.810 | 2.640 | 2.154 |
| 6 | | | 2.694 | 2.499 | 1.359 | 1.561 |
| 7 | | | 2.408 | 2.481 | 0.347 | 1.390 |
| 8 | | | 2.176 | 1.970 | 1.624 | 0.865 |
| 9 | | | 1.461 | 1.422 | 0.887 | 0.604 |
| 10 | | | 1.286 | 0.926 | 0.543 | 0.519 |
| 11 | | | 0.981 | 0.781 | 0.294 | 0.294 |
| 12 | | | 0.812 | 0.650 | 0.249 | 0.199 |
| 13 | | | 0.373 | 0.432 | 0.239 | 0.209 |
| 14 | | | 0.653 | 0.371 | 0.145 | 0.184 |
| 15 | | | 0.441 | 0.348 | 0.151 | 0.151 |
| 16 | | | 0.321 | 0.374 | 0.098 | 0.106 |
| 17 | | | 0.525 | 0.186 | 0.099 | 0.108 |
| 18 | | | 0.351 | 0.171 | 0.083 | 0.090 |
| 19 | | | 0.192 | 0.164 | 0.084 | 0.087 |

23 10 99 57:27

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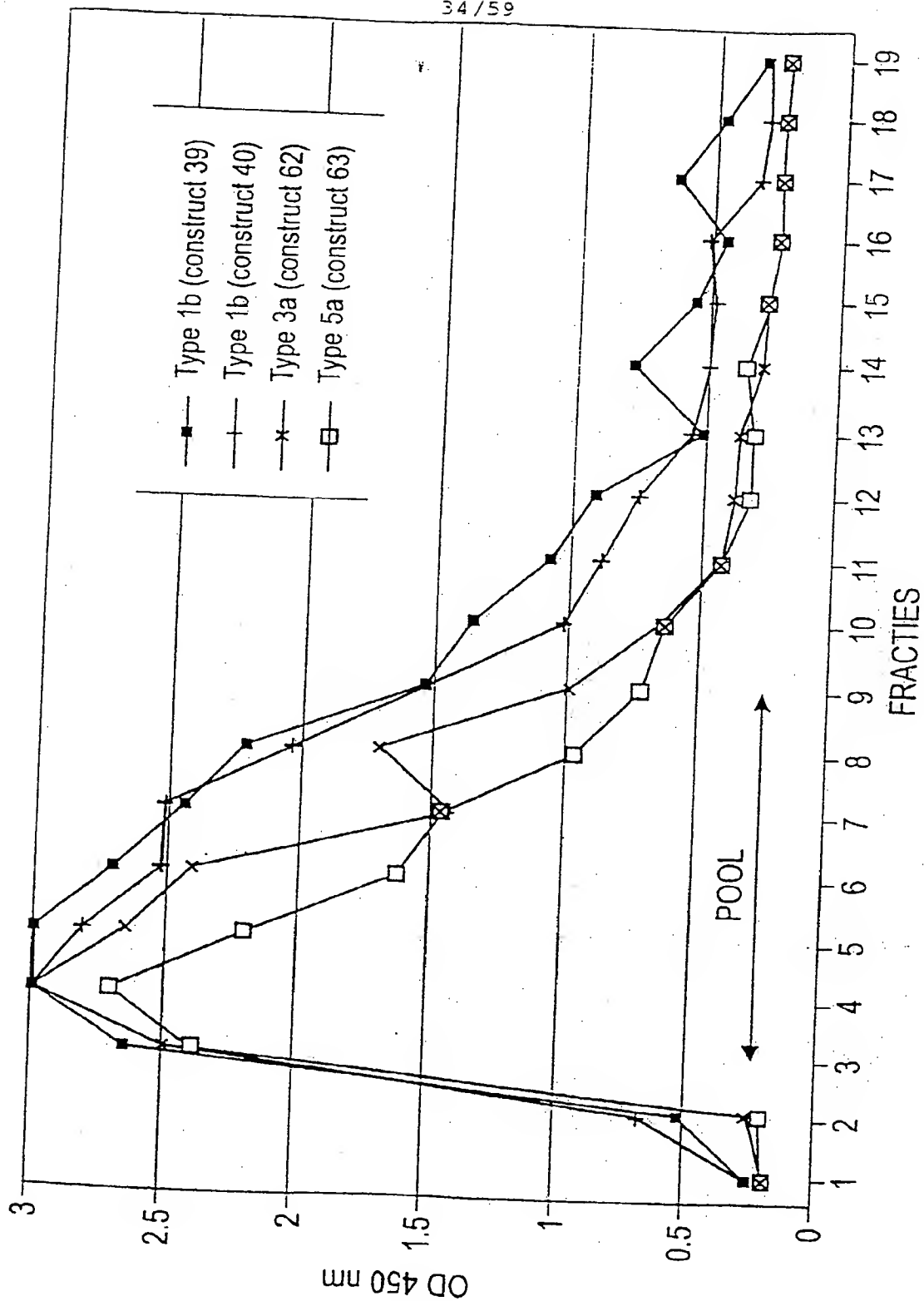


FIGURE 23

35/59 Figure 24

| Fraction | volume | dilution | OD measured at 450 nm | | | |
|----------|-------------|----------|-----------------------|------------------|------------------|------------------|
| | | | construct | | | |
| | | | 39 Type 1b | 40 Type 1b | 62 Type 3a | 63 Type 5a |
| 20 | 250 μ l | 1/200 | 0.072 | 0.130 | 0.096 | 0.051 |
| 21 | | | 0.109 | 0.293 | 0.084 | 0.052 |
| 22 | | | 0.279 | 0.249 | 0.172 | 0.052 |
| 23 | | | 0.093 | 0.151 | 0.297 | 0.054 |
| 24 | | | 0.080 | 0.266 | 0.438 | 0.056 |
| 25 | | | 0.251 | 0.100 | 0.457 | 0.048 |
| 26 | | | 3 | 1.649 | 0.722 | 0.066 |
| 27 | | | 3 | 3 | 2.528 | 0.889 |
| 28 | | | 3 | 3 | 3 | 2.345 |
| 29 | | | 3 | 3 | 2.849 | 2.580 |
| 30 | | | 2.227 | 1.921 | 1.424 | 1.333 |
| 31 | | | 0.263 | 0.415 | 0.356 | 0.162 |
| 32 | | | 0.071 | 0.172 | 0.154 | 0.064 |
| 33 | | | 0.103 | 0.054 | 0.096 | 0.057 |
| 34 | | | 0.045 | 0.045 | 0.044 | 0.051 |
| 35 | | | 0.043 | 0.047 | 0.045 | 0.046 |
| 36 | | | 0.045 | 0.045 | 0.049 | 0.040 |
| 37 | | | 0.045 | 0.047 | 0.046 | 0.048 |
| 38 | | | 0.046 | 0.048 | 0.047 | 0.057 |
| 39 | | | 0.045 | 0.048 | 0.050 | 0.057 |
| 40 | | | 0.046 | 0.049 | 0.048 | 0.049 |

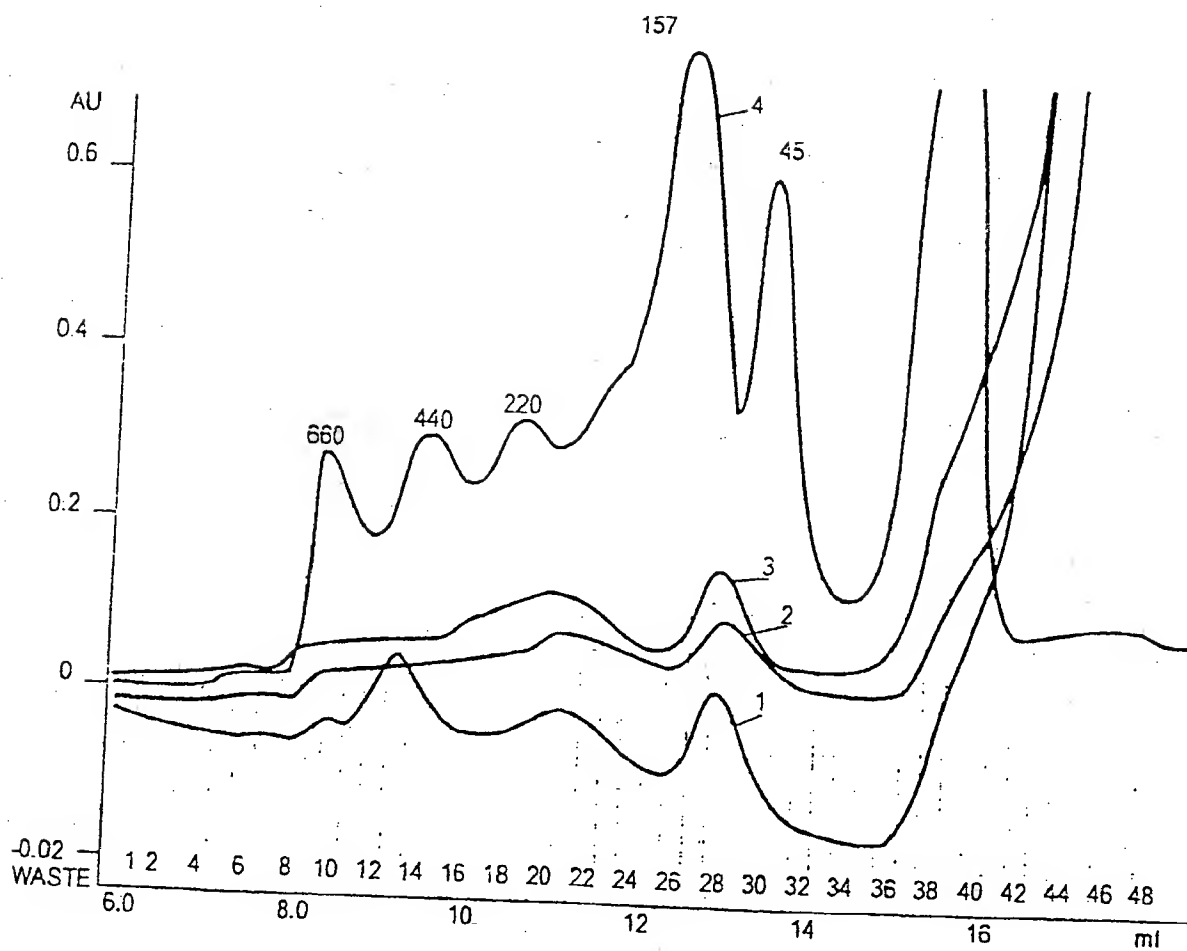


FIGURE 25

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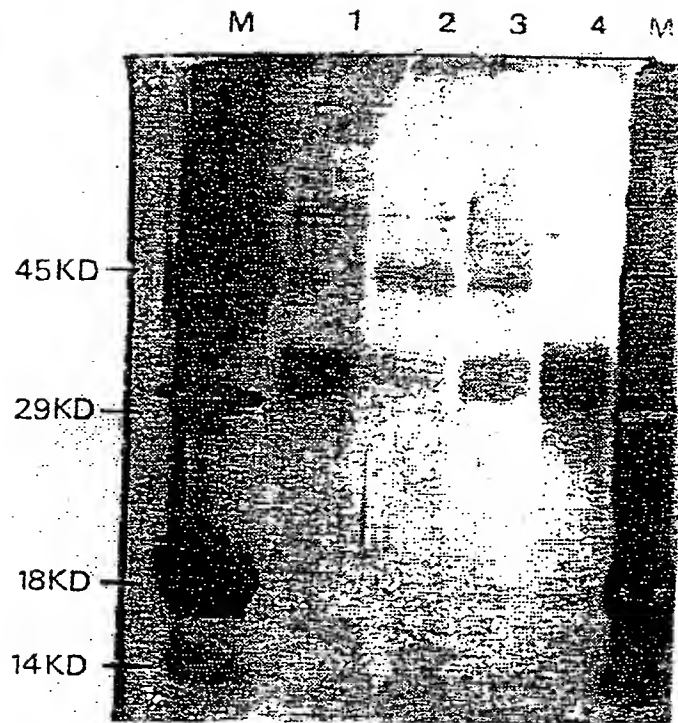


Figure 26

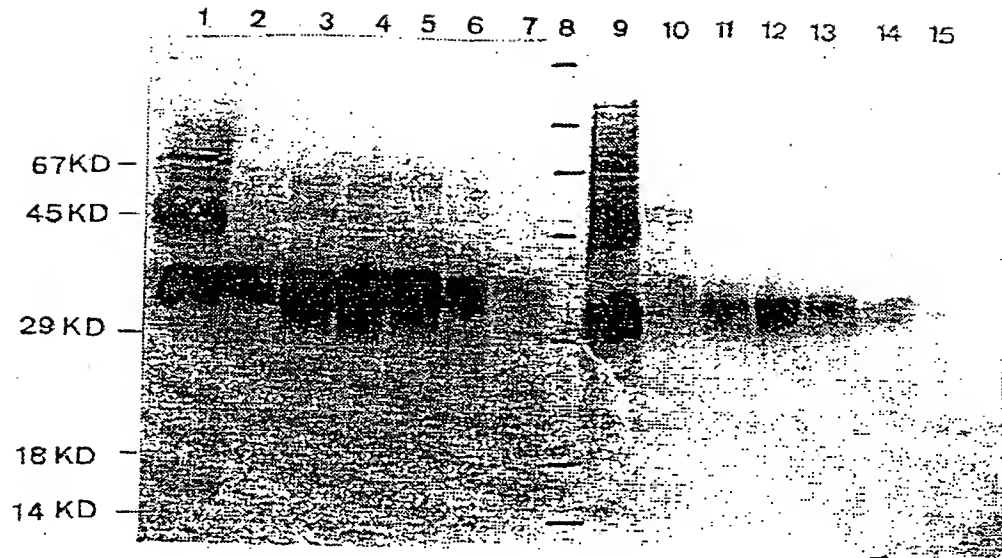


Figure 27

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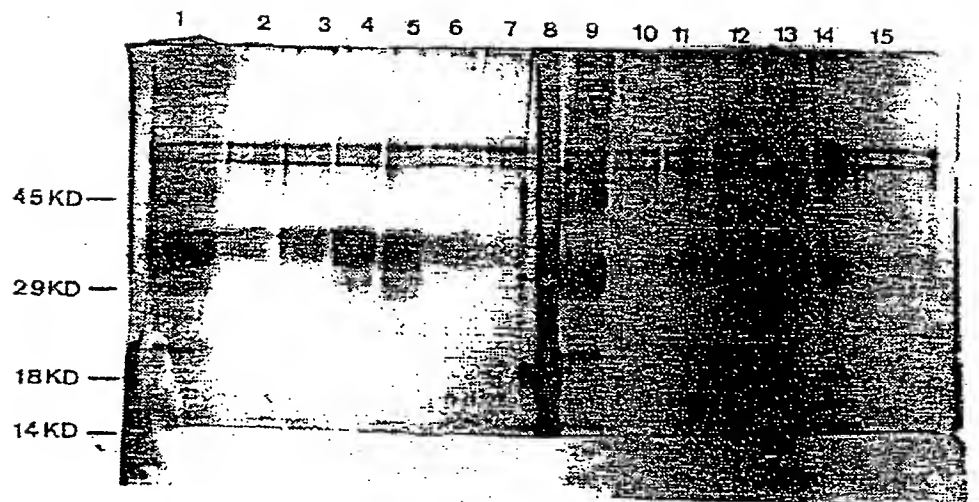
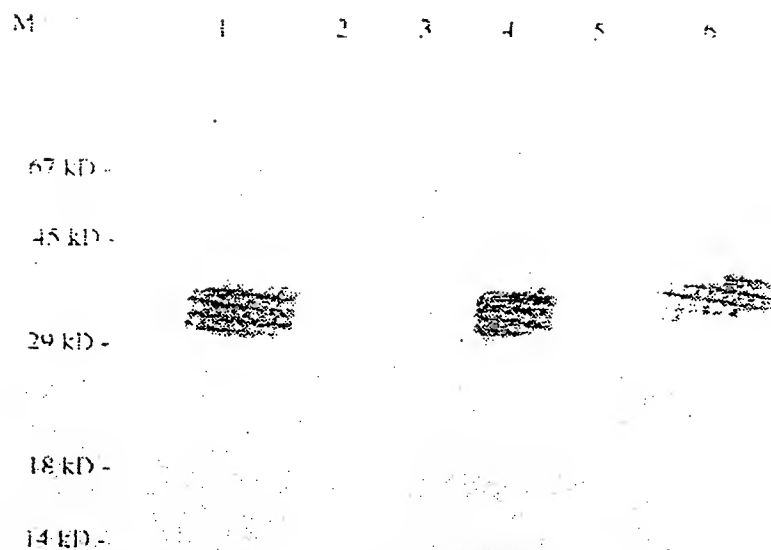


Figure 28



Lane 1: Crude Lysate
Lane 2: Flow through Lentil Chromatography
Lane 3: Wash with EMPIGEN Lentil Chromatography
Lane 4: Eluate Lentil Chromatography
Lane 5: Flow through during concentration lentil eluate
Lane 6: Pool of E1 after Size Exclusion Chromatography

Figure 29: Western Blot Analysis with anti-E1 mouse monoclonal 5E1A10

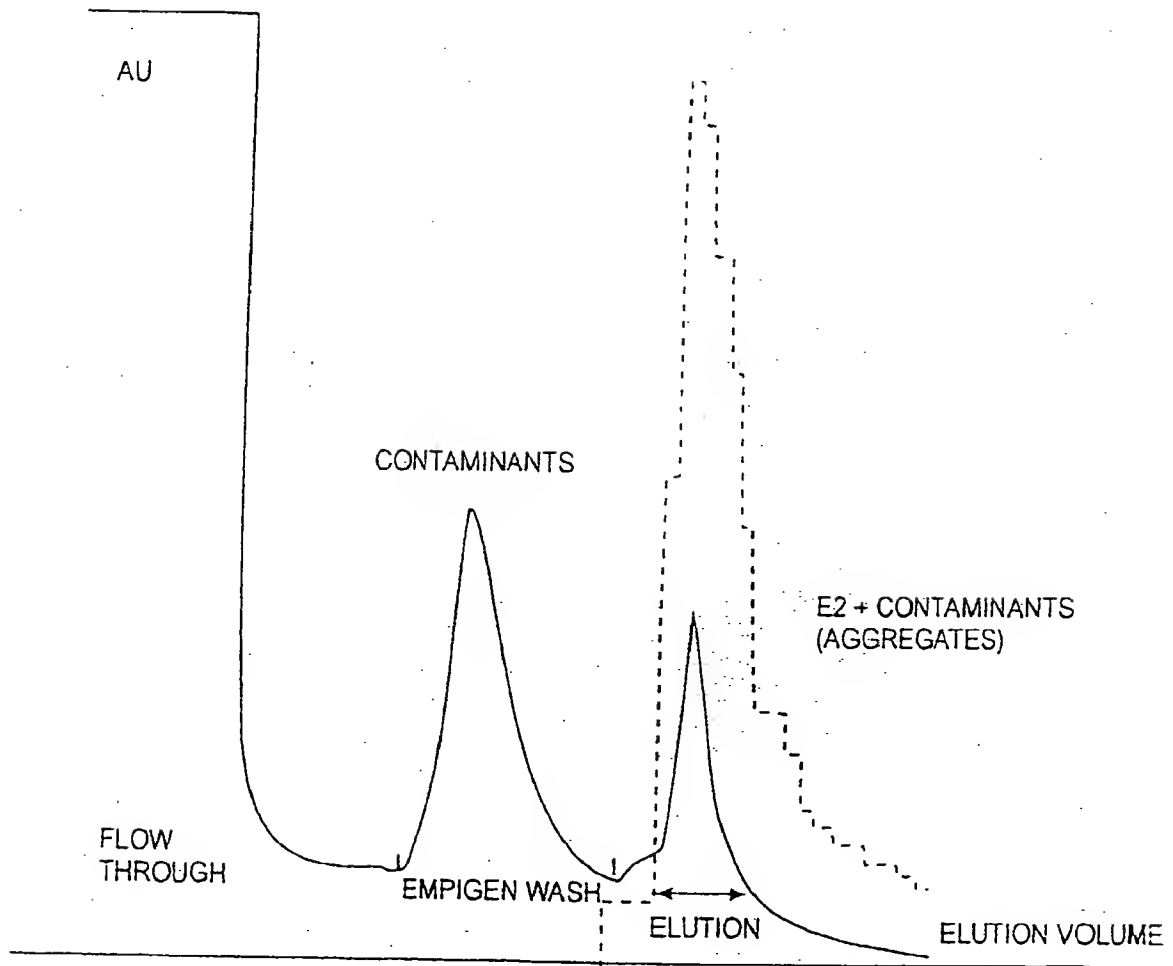
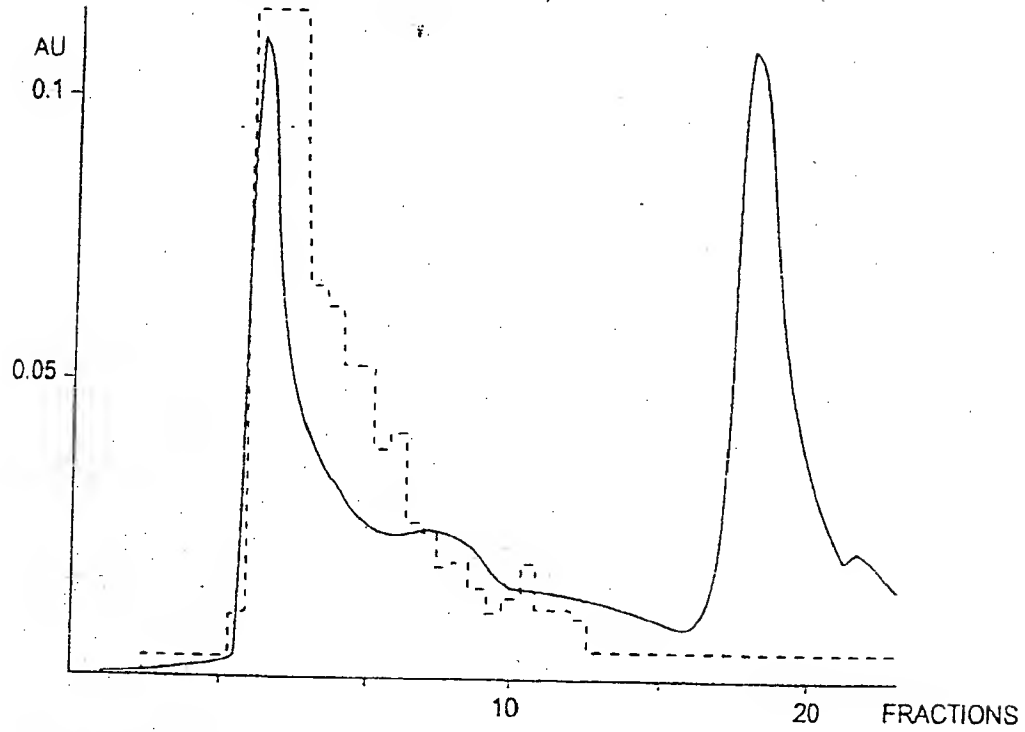


FIGURE 30

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A: NON - REDUCED

E2 + CONTAMINANTS (AGGREGATES)



B: REDUCED

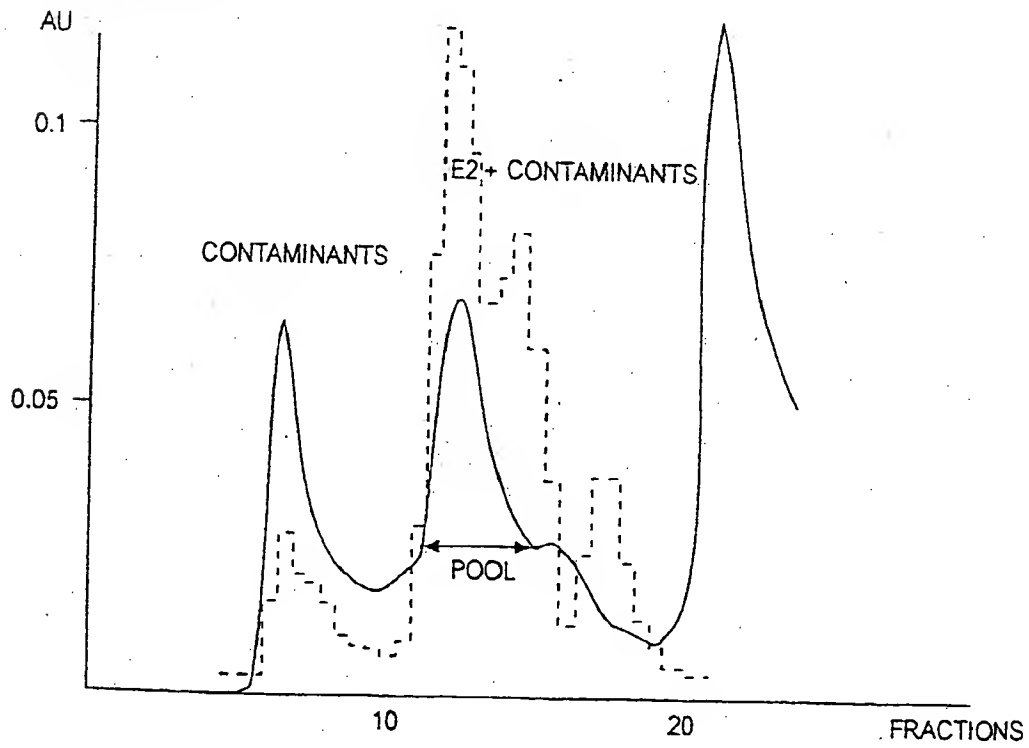


FIGURE 31

20 10 00 5100

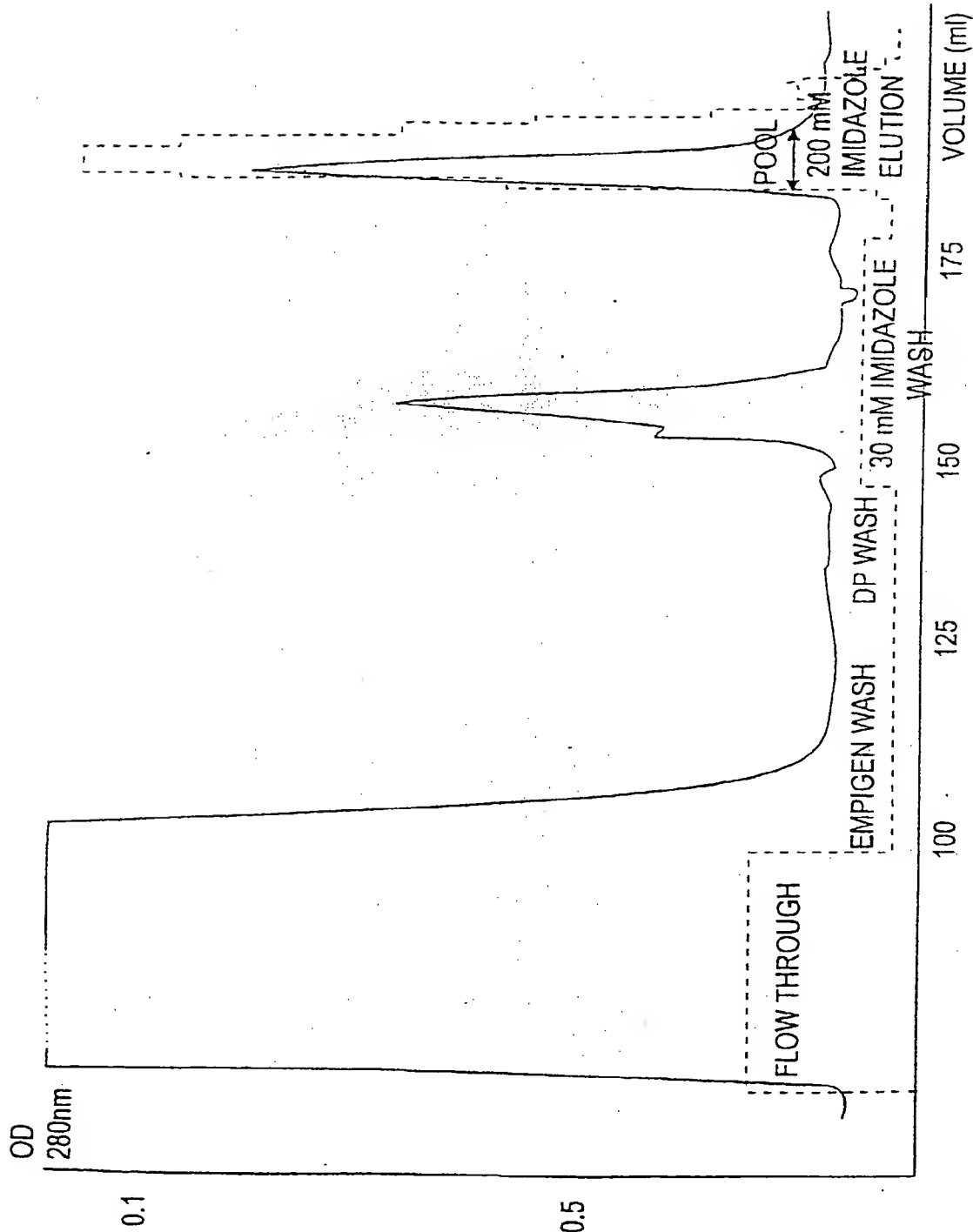
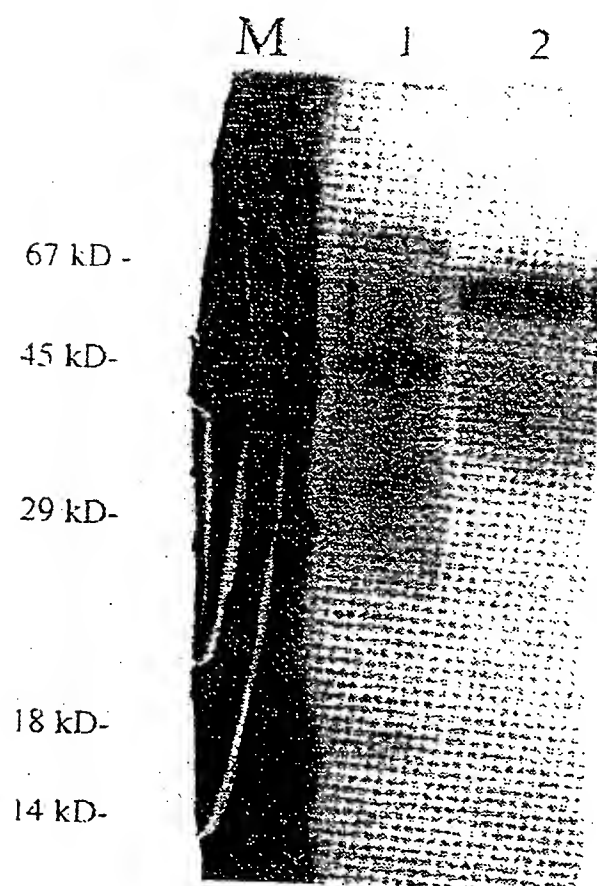
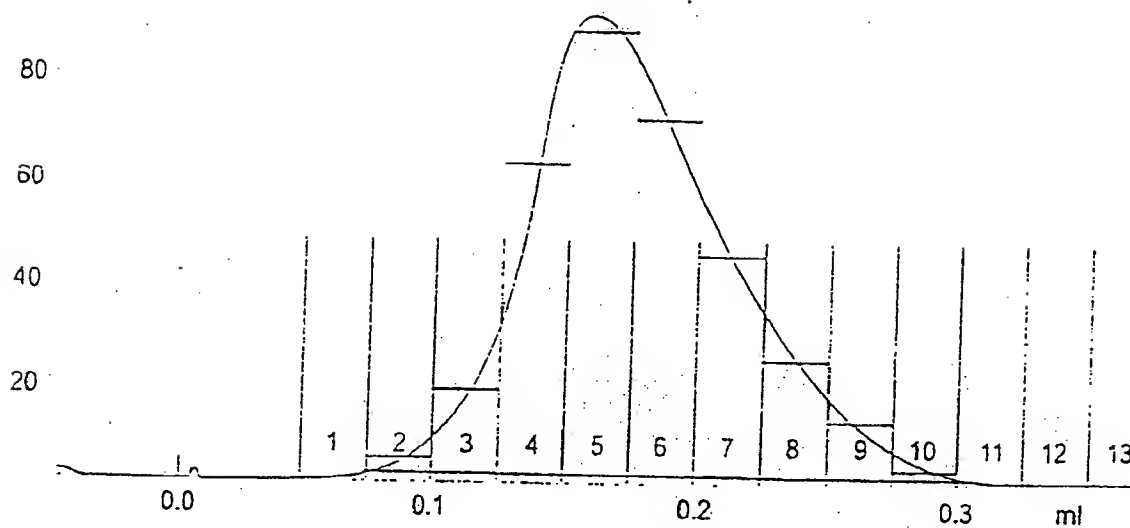


FIGURE 32

**FIGURE 33:
SILVER STAIN OF PURIFIED E2**



1. 30 mM IMIDAZOLE WASH Ni-IMAC
2. 0.5 ug E2



| No. | Ret. (ml) | Peak start (ml) | Peak end (ml) | Dur (ml) | Area (ml*mAU) | Height (mAU) |
|-----|--------------|--------------------|------------------|-------------|------------------|-----------------|
| 1 | -0.45 | -0.46 | -0.43 | 0.04 | 0.0976 | 4.579 |
| 2 | 1.55 | 0.75 | 3.26 | 2.51 | 796.4167 | 889.377 |
| 3 | 3.27 | 3.26 | 3.31 | 0.05 | 0.0067 | 0.224 |
| 4 | 3.33 | 3.32 | 3.33 | 0.02 | 0.0002 | 0.018 |

Total number of detected peaks = 4

Total Area above baseline = 0.796522 ml*AU

Total area in evaluated peaks = 0.796521 ml*AU

Ratio peak area / total area = 0.999999

Total peak duration = 2.615583 ml

FIGURE 35A

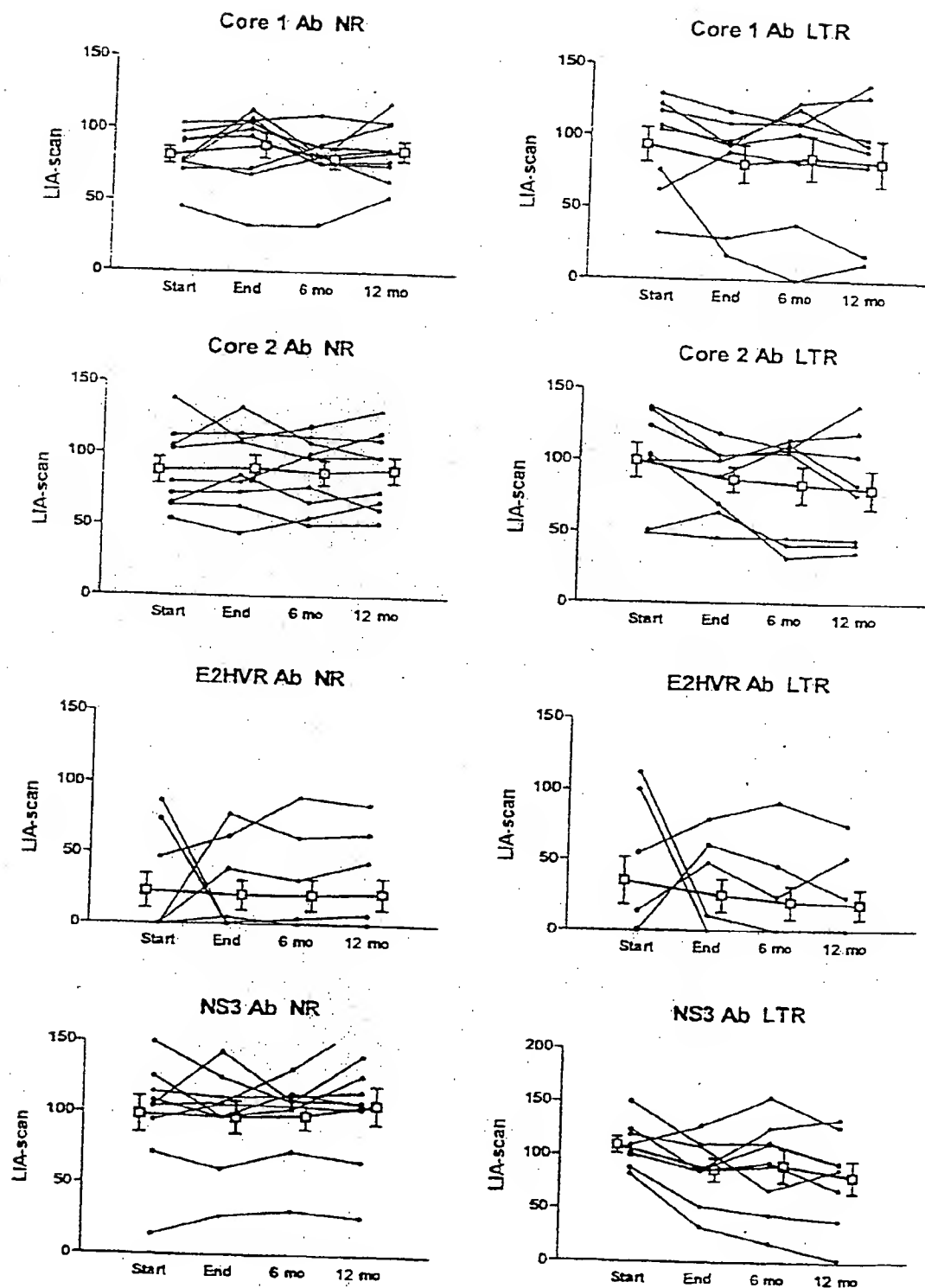
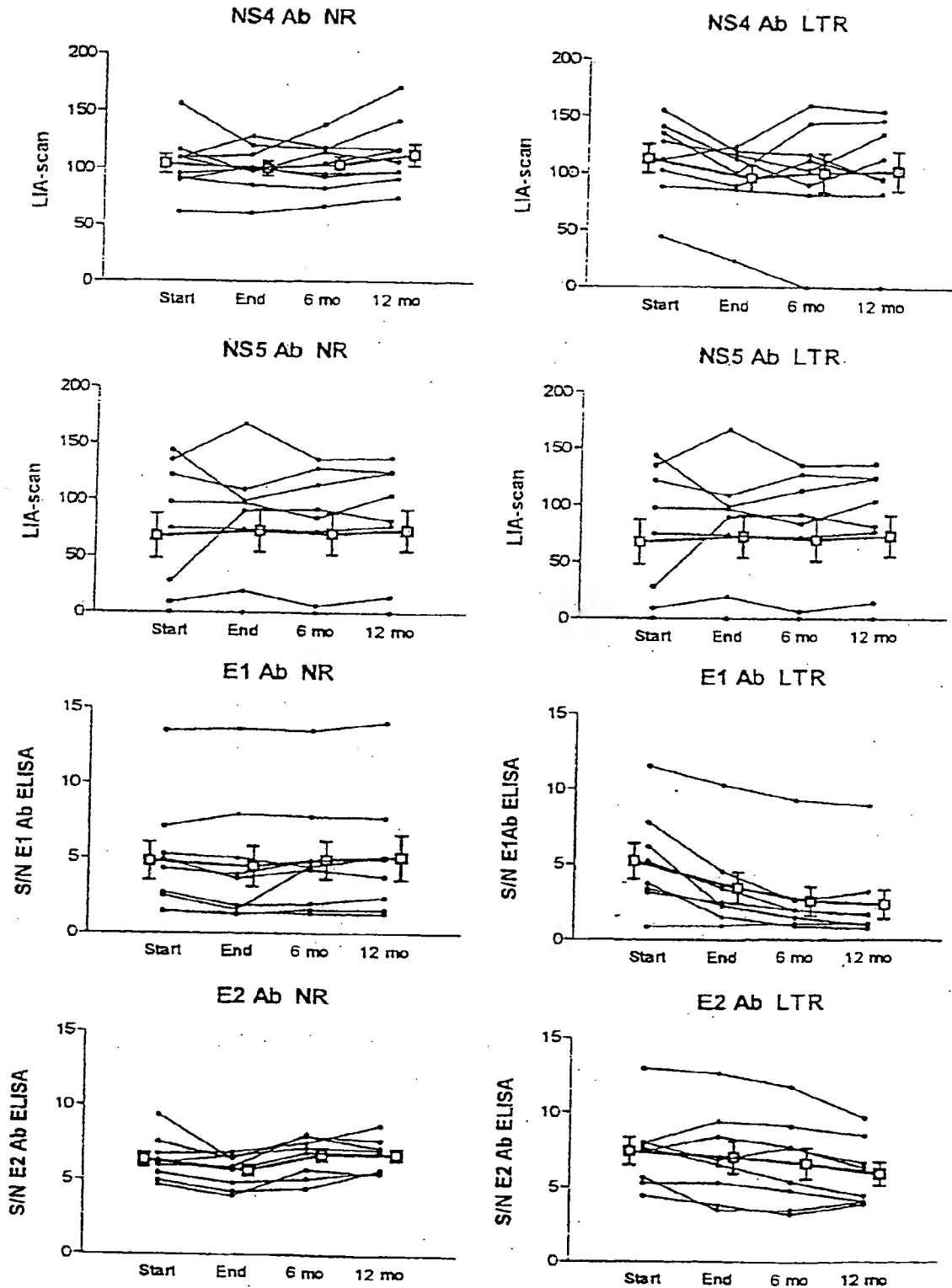
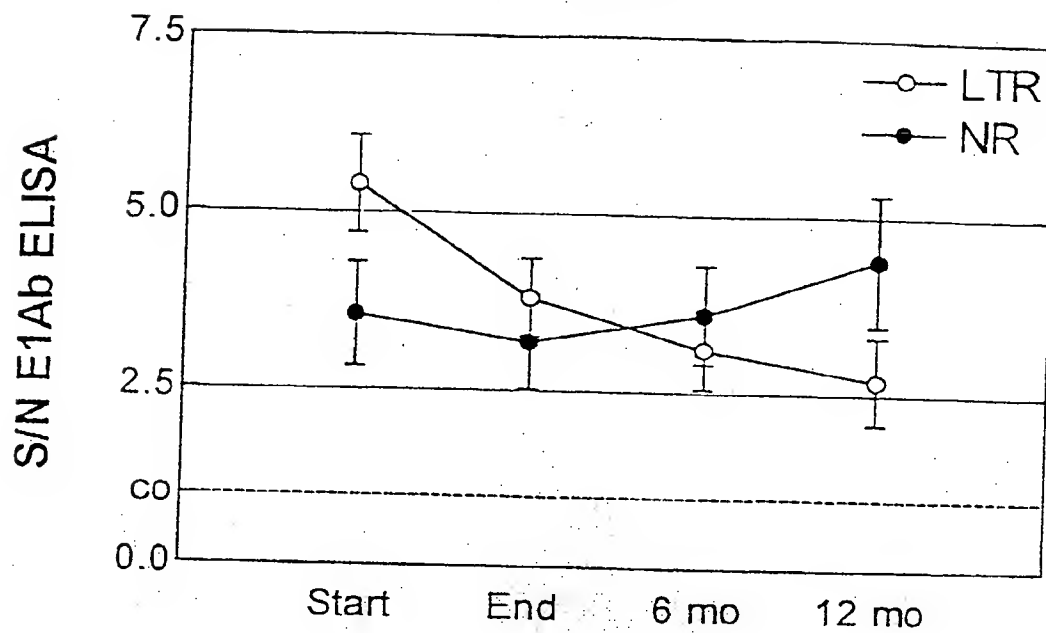


FIGURE 35B



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Figure 36

E1 Ab



E2 Ab

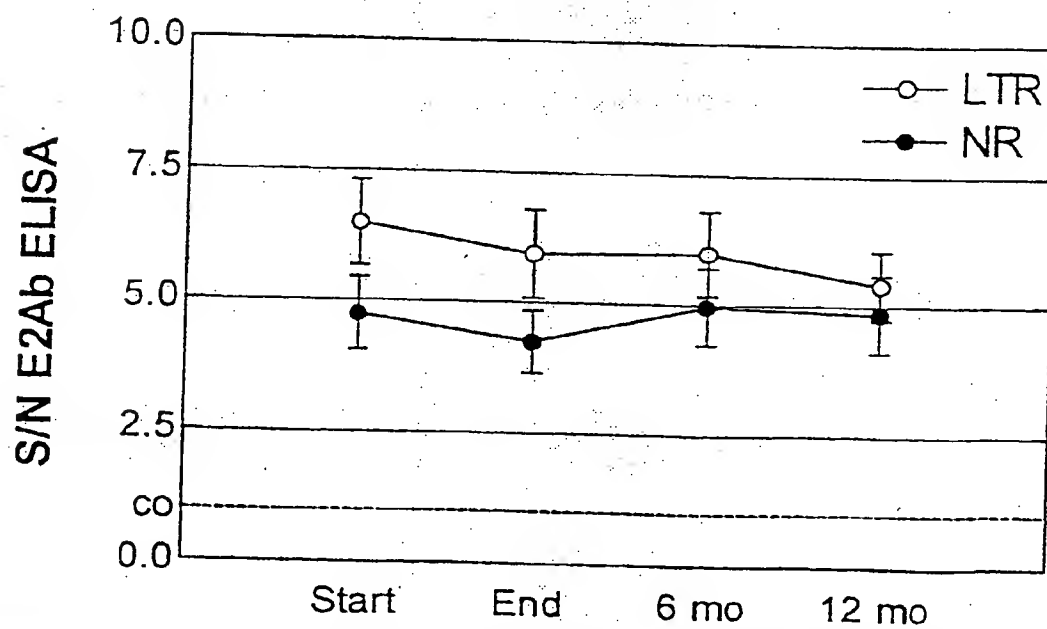


FIGURE 37

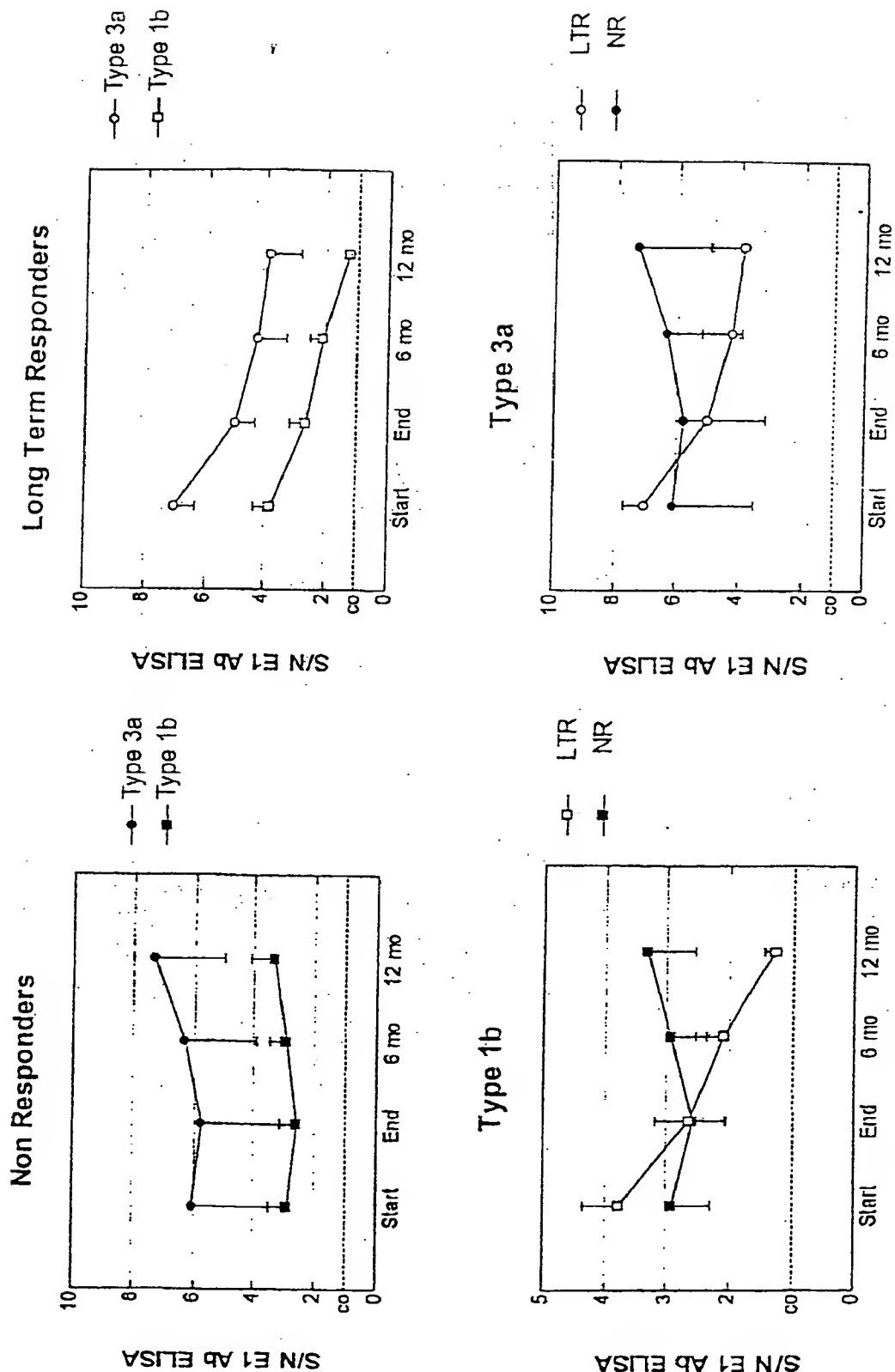
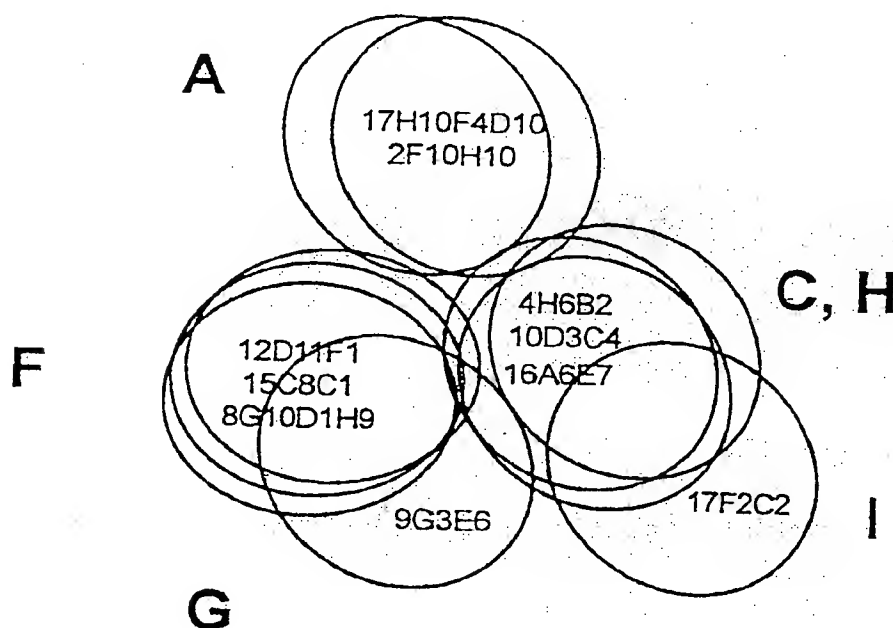


Figure 38

Relative Map Positions of
anti-E2 monoclonal antibodies

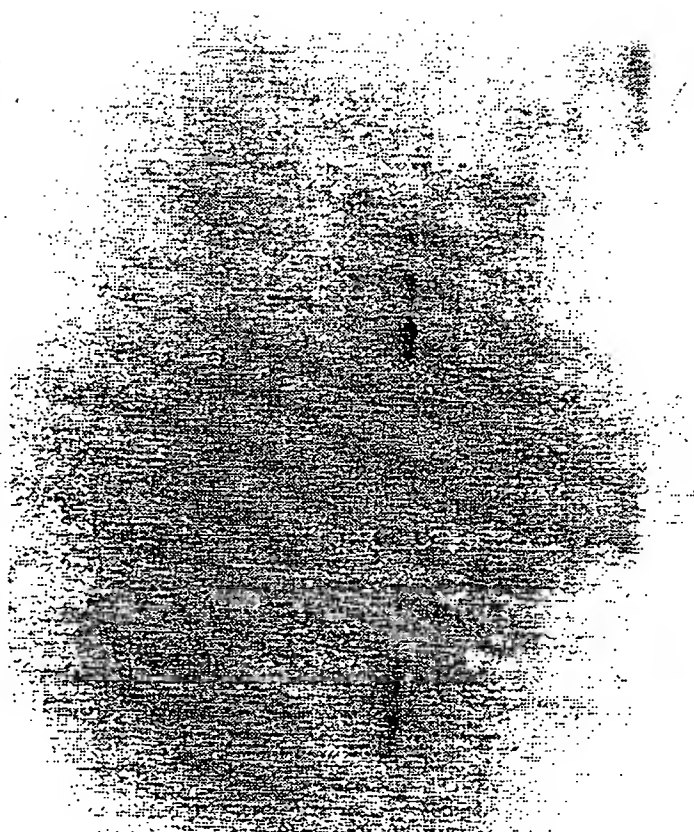


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PARTIAL DEGLYCOSYLATION OF HCV E1 ENVELOPE PROTEIN

| Endoglycosidase H (Endo H) | Glycopeptidase F (PNGase F) |
|-------------------------------|--------------------------------|
| 0µg | 0µg |
| 0.6µg | 0.04µg |
| 6µg | 4µg |
| 60µg | 40µg |
| 0.6mg | 400µg |

0µg 0.6µg 6µg 60µg 0.6mg 0µg 0.04µg 4µg 40µg 400µg



↑
↑
↑
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- 106.0
- 80.0
- 49.5
- 32.5
- 27.5
- 18.5

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Figure 39

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PARTIAL TREATMENT OF HCV E2\ E2s ENVELOPE PROTEINS BY PNGase F

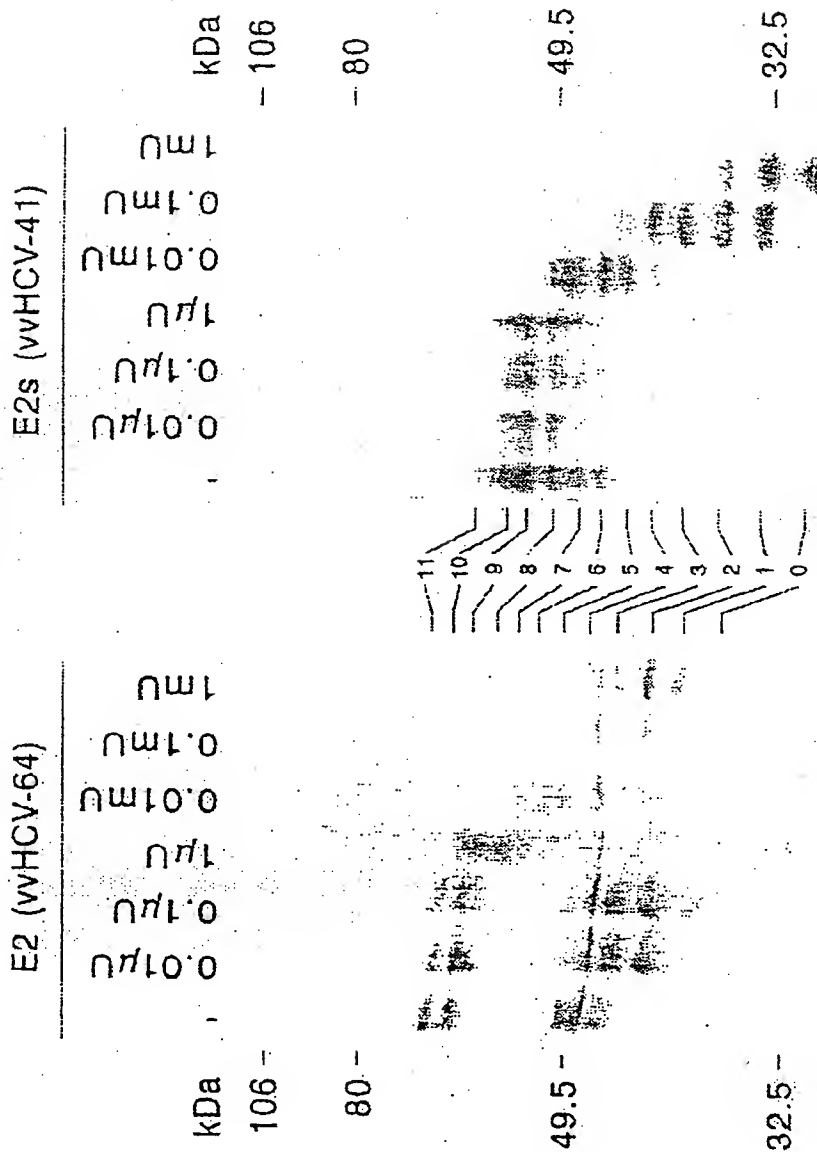
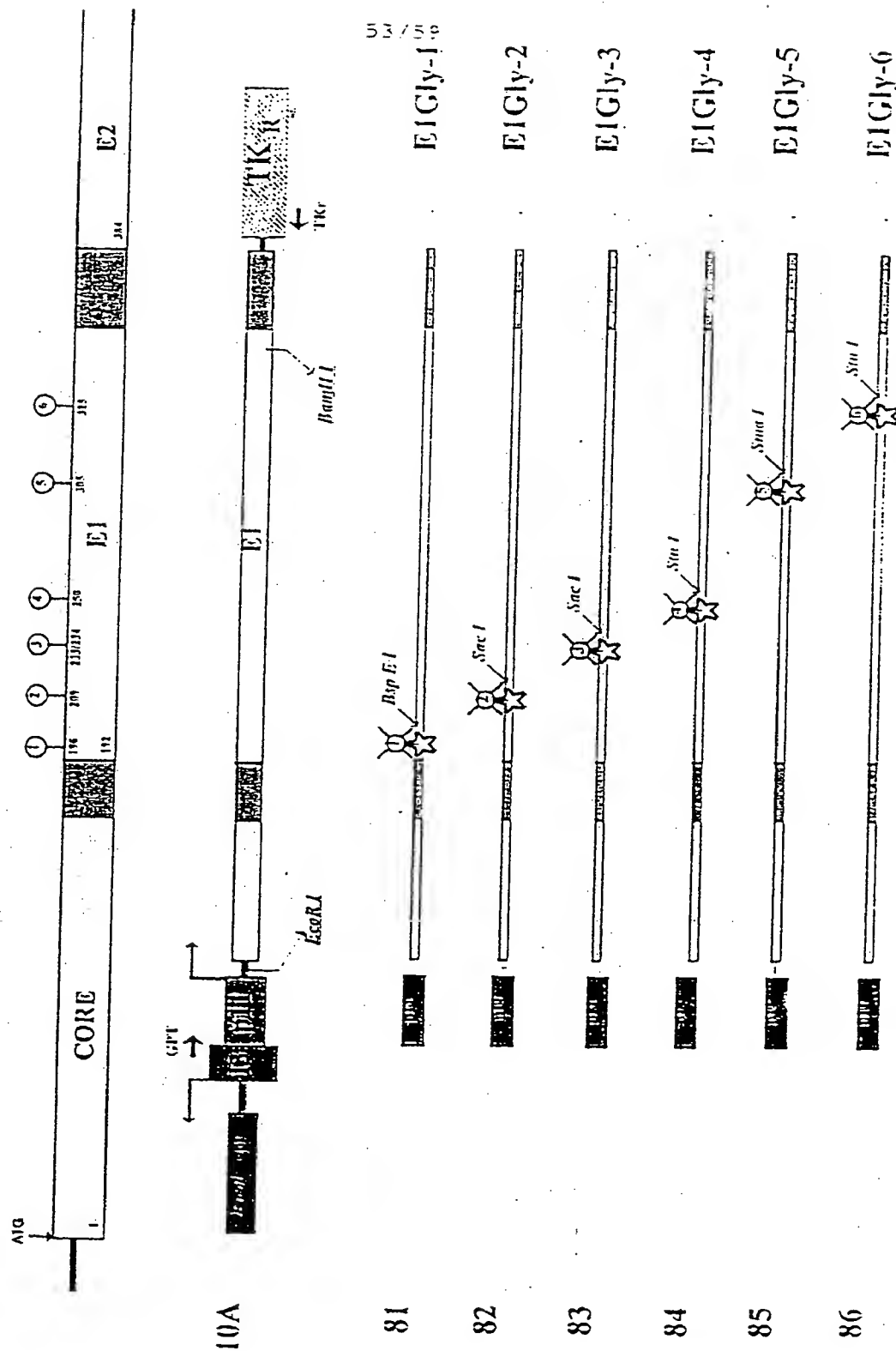


Figure 40

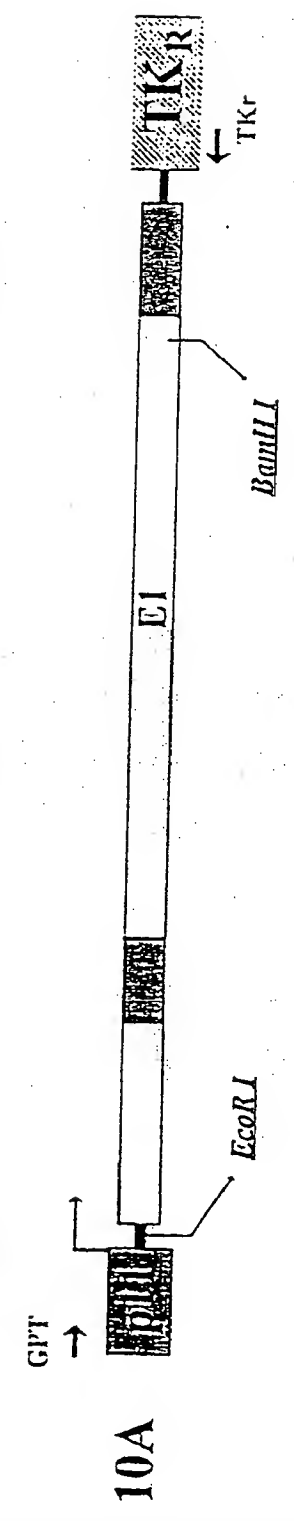
Fig. 41

In Vitro Mutagenesis of HCV E1 glycoprotein

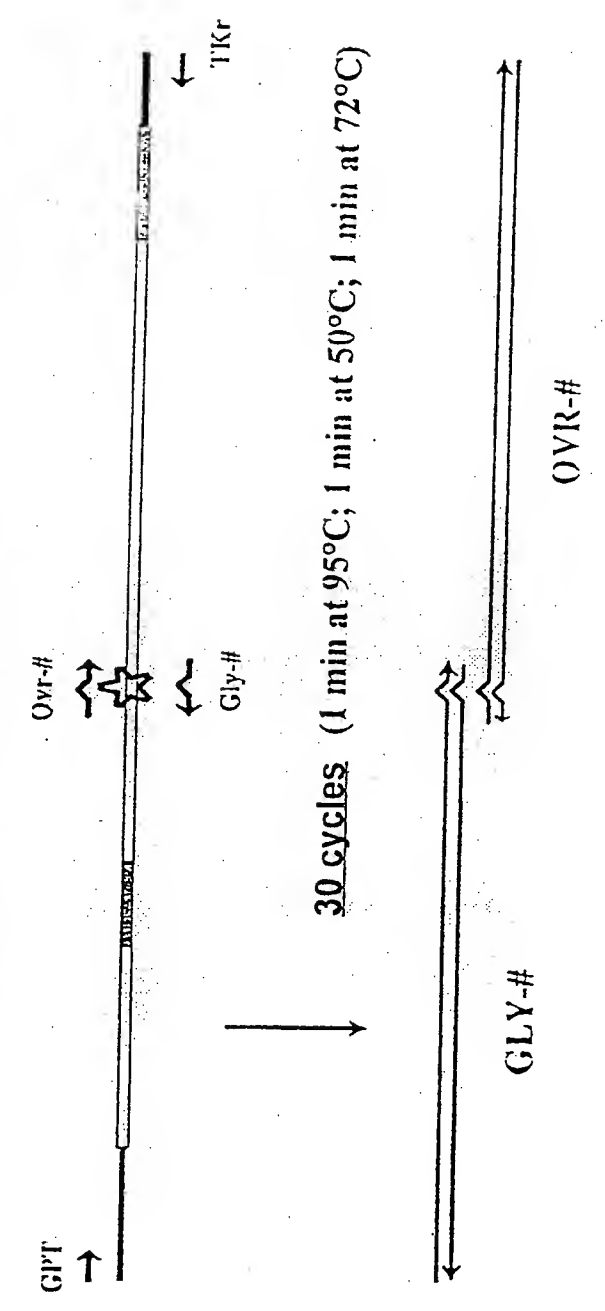


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Fig. 42A In Vitro Mutagenesis of HCV E1 glycoprotein



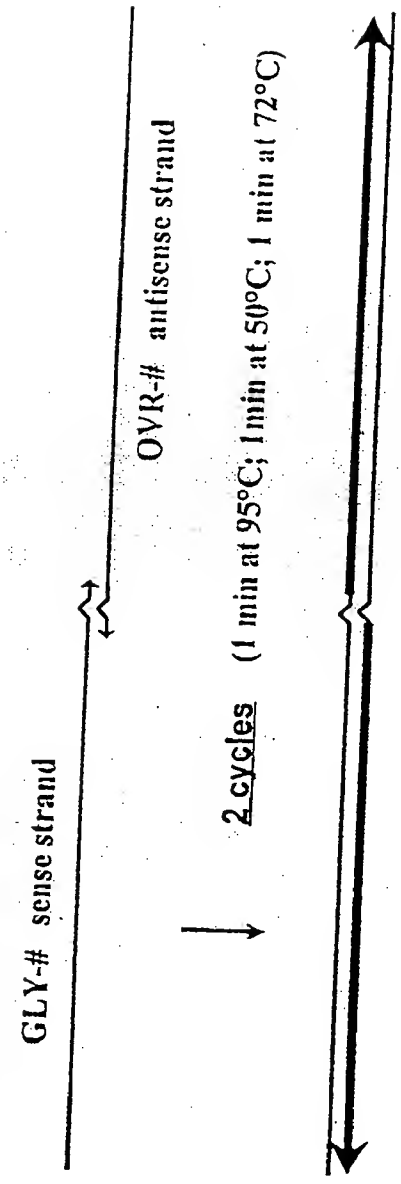
1. First step of PCR amplification (Gly-# and Ovr-# primers)



2. Overlap extension and nested PCR

Fig. 42B

a. Overlap extension



b. Nested PCR amplification (GPT-2 and TKR-2 primers)

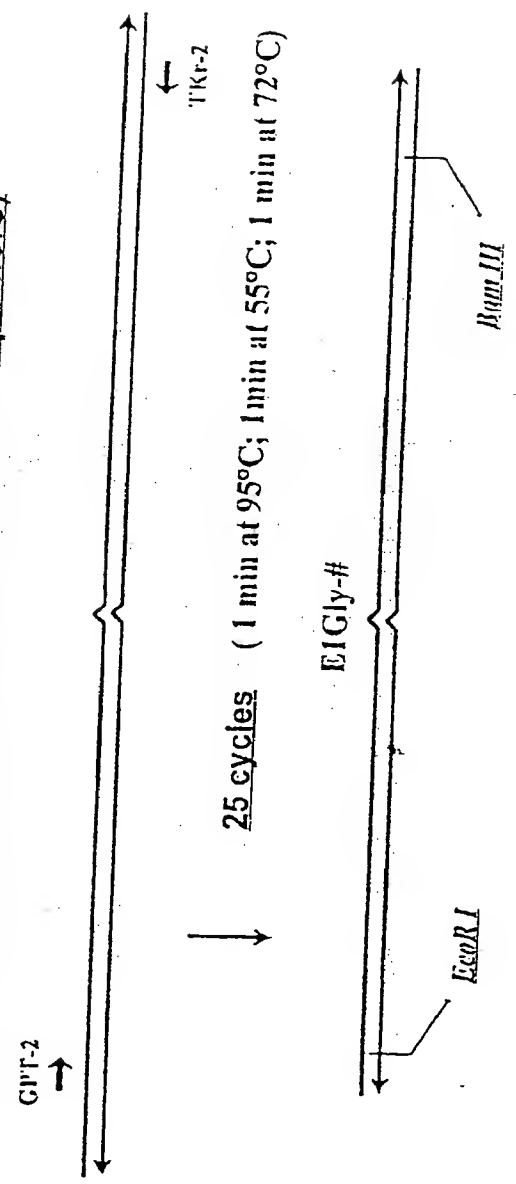
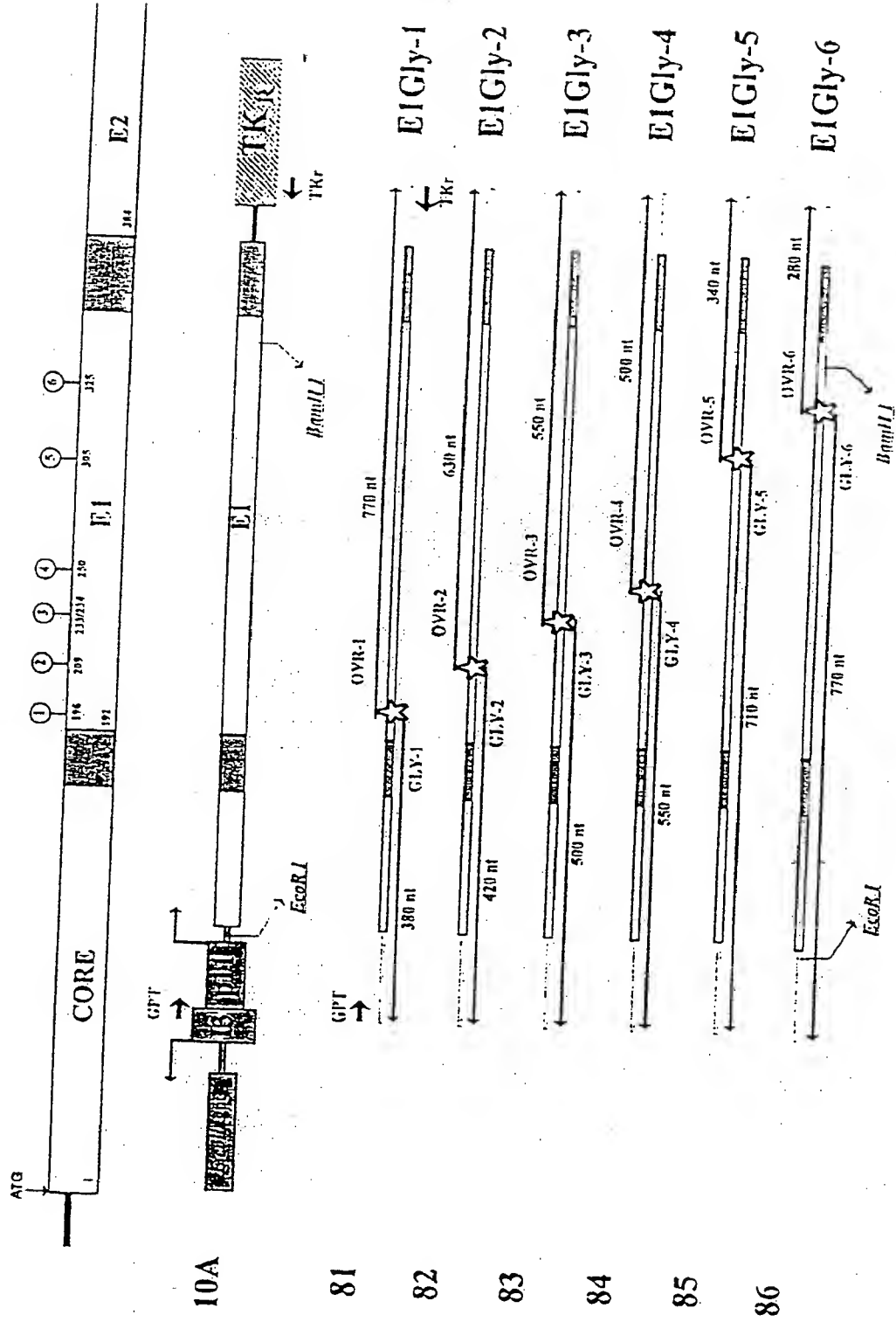


Fig. 43 *In Vitro* Mutagenesis of HCV E1 glycoprotein



| HeLa cells | | | | | | | RK 13 cells | | | | | | | | | | |
|------------|-------|---|---|---|---|---|-------------|------|-------|---|---|---|---|---|---|---|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | 2 | 1 | 3 | 4 | 5 | 6 | 7 | 8 | |
| 80.0 | ----- | | | | | | | 80.0 | ----- | | | | | | | | 80.0 |
| 49.5 | | | | | | | | 49.5 | | | | | | | | | 49.5 |
| 32.5 | | | | | | | | 32.5 | | | | | | | | | 32.5 |
| 27.5 | | | | | | | | 27.5 | | | | | | | | | 27.5 |
| 18.5 | | | | | | | | 18.5 | | | | | | | | | 18.5 |

Figure 44A

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Figure 44B

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Figure 45

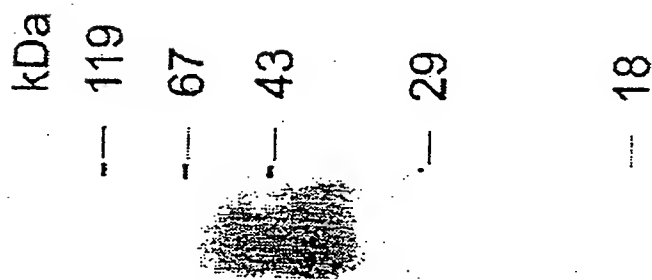


Figure 46

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